Уральский федеральный университет
имени первого Президента России Б.Н.Ельцина
Химико-технологический институт

Е. Г. КОВАЛЕВА
С. Ю. МИТРОПОЛЬСКАЯ

# БЕЗОПАСНОСТЬ И КАЧЕСТВО ПИЩЕВЫХ ПРОДУКТОВ

# PRACTICAL FOOD SAFETY AND FOOD QUALITY

Практикум

МИНИСТЕРСТВО ОБРАЗОВАНИЯ И НАУКИ РОССИЙСКОЙ ФЕДЕРАЦИИ
УРАЛЬСКИЙ ФЕДЕРАЛЬНЫЙ УНИВЕРСИТЕТ
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Е. Г. Ковалева
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# PRACTICAL FOOD SAFETY AND FOOD QUALITY

Практикум

Рекомендовано методическим советом УрФУ для студентов,
обучающихся по программе магистратуры
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информационных технологий, механики и оптики
(заведующий кафедрой доктор технических наук А. В. Ф е д о р о в);
Е. Н. Ш а р а ф у т д и н о в а, кандидат химических наук, доцент,
доцент кафедры управления качеством
(Уральский государственный экономический университет)
Н а у ч н ы й р е д а к т о р
М. А. М и р о н о в, доктор химических наук, профессор

**Ковалева, Е. Г.**

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Даны описания практических и лабораторных работ, проводимых в рамках курсов «Международные стандарты и безопасность продуктов питания» и «Контроль качества биотехнологических продуктов». Задания сопровождаются подробными комментариями. Используются активные формы обучения, такие как работа в команде, игровые технологии и пр.

Для иностранных и российских студентов, обучающихся на английском языке, изучающих пищевую биотехнологию, контроль качества и безопасность пищевых продуктов.

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# ОГЛАВЛЕНИЕ

# PREFACE

Tutorials and laboratory experiments play a very important role in a learning process. The main objective of a tutorial is to consolidate the knowledge/information acquired in the corresponding lecture. A tutorial seeks to teach by example and supply the information to complete a certain task and is more interactive and specific than a book or a lecture.

The aim of laboratory work is to deepen and fix theoretical knowledge and to develop the skills of independent experimentation.

This book is aimed to provide the Master's students of Food Biotechnology an option with necessary guidelines for tutorials and laboratory experiments on food safety and analysis in the course "International Standards in Biotechnology and Food Safety" and "Quality Control of Biotechnological Products". It is organized into two chapters with a description of 8 tutorials and 8 laboratory experiments related to food safety and quality control analysis. Each typical subchapter consists of backgrounds, tutorial task or activity, discussion points and references.

The tutorials and laboratory experiments developed and presented in the book are adapted to the continuous technical and scientific training tasks. In addition, some of them actively employ methods for the modern educational technologies, such as team work, quiz show and research method, other contain special tutorial's tasks which are given to students.

In the end of the book the list of recommended literature and resources is given for additional self-studies for preparation for Tutorials and Laboratory experiments.

Authors are very grateful to the book's reviewers for the comments and recommendations made in the process of its preparation.

# INTRODUCTION

*Food safety* and *food quality* is a vast subject area.

*Food safety* is a complex problem that requires many efforts to solve it, both from the community of scientists, biochemists, microbiologists, toxicologists, etc., and from the manufacturers of sanitary-and-epidemiologic institutions, governmental authorities and, finally, consumers.

*The topicality of food safety* as a problem increases every year, since the safety of raw materials and food stuffs is one of the main factors affecting peoples' health and the genetic conservation.

*Food safety* means non dangerous to human health consumption of food stuffs both in terms of acute negative effects (food poisoning and food infection), and from the point of view of the danger of long-term effects (carcinogenic, mutagenic and teratogenic effects). in other words, safe food is food stuffs which does not cause adverse effects on the health of present and future generations.

Considerable amounts of hazardous substances to human health, such as xenobiotics of chemical (toxic elements, pesticides, dioxins, nitric compounds, hormones, histamine, polyaromatic carbon hydrogens, polychlorated biphenyls, radionuclides etc.) and biological origin (pathogenic microorganisms, viruses, mycotoxins, antibiotics, helminth and protozoa etc.), as well as genetically modified sources (GMS) enter human body with food. Therefore, we are currently facing the acute problems which are associated with an increased responsibility for the efficiency and objectivity of food control to ensure food stuffs safety for the consumers' health.

*Food quality*, in its turn, is not limited to either the organoleptic quality enjoyed by the consumer or bacteriological and chemical examination. *Food quality control* is a complex area which uses the sum of all those controllable factors that ultimately influence positively or negatively the quality of the finished products, e.g. selection

of raw materials, processing methods, packaging, methods of storage and distribution, etc.

There is a number of research methods for qualitative and quantitative analysis of food stuffs which are currently widely used for food stuffs. Among them there can be mentioned gas chromatography (GS) and high performance liquid chromatography (HPLC), atomic absorption spectrometry (AAS), fluorescence spectrophotometry (FS), capillary electrophoresis, infrared spectroscopy (IR), electrochemical methods, EPR spectroscopy, classical methods of analysis such as titrimetric, gravimetric and rheological methods of research. Various versions of the chain reaction method (PCR) are used the qualitative and quantitative measures of genetic-modified sources, the enzyme-linked immunosorbent assay (ELISA) is applied to analysis of antibiotics in food stuffs, whereas bacteriological control is performed by routine microbiological analysis.

Tutorials and laboratory experiments presented in this book are designed for detailed acquaintance of Master's students in an area of Food Biotechnology with International standards for food stuffs, different aspects in quality control of beer and wine, dairy products, coffee, sweet soft drinks and meat-based stuffs, food additives and biologically active substances, methods for detection and analysis of xenobiotics of chemical and biological origin and genetically modified sources (GMS) of plant origin in food stuff and raw materials, food microbiological analysis, as well as to food irradiation and packaging technologies.

Each tutorial gives practical experience related to the corresponding lecture, and helps students implementing the acquired knowledge in a laboratory research activity, as well as in setting up the independent research project, writing and defending the graduation thesis.

The tutorials and laboratory works are mentored by either a lecturer or a tutor. the tutorial leader usually gives a short (10–15 minutes) introduction in order to refresh the lecture content and warm up a student for the team-work. Prior to the tutorial, the students study the basic topics of a tutorial in details searching for the information from the textbooks/Internet or their own experience in the labs. During self-learning

the students can check their understanding of the basic topics of a tutorial with discussion points. the students are supposed to raise and lead their own discussions within the group among each other under supervision of a mentor (lecturer/tutor). the supervisor must thoroughly follow the course of the discussion and correct it in case of any necessity. Upon the desire, the students prepare short Power Point presentation.

Laboratory-based works must be carried out in specialized biotechnological rooms, equipped with ventilation, laminar flow cabinets' wardrobes, sanitation, tanks for drains collection. to succeed in laboratory experiments the students should be well-prepared, i.e. self-study lecture notes as well as the recommended academic literature, be able to take part in the discussion of the experiments which they are going to carry out. After the experiments performed, the students are required to submit their reports.

# Chapter 1
# TUTORIALS

## Tutorial 1. International Standards and Regulations Related to Food Analysis

**Basic Topics for Discussion**

1. The system of quality control of products at the international level.
2. International standards, methodological fundamentals and modern development in quality management.
3. HACCP and ISO systems.
4. International Standards and Regulations Related to Food Analysis.

**Tutorial Task "Analysis of a Standard: a Typical Layout of Codex Alimentarius Standards"**

*Students are supposed to*

- Read attentively a Codex Alimentarius Standard on food products provided.
- What are the main structural parts/clauses of the standard?
- Do they contain any sub-clauses? What kind of requirements do they stipulate?
- How does this standard influence suppliers of food products?
- Fill-in the following form of Report (*Annex 1*).

An Optional List of Standards to Be Provided (may be reconsidered subjected to students' background), for instance,

CODEX STAN 182-1993 CODEX STANDARD FOR PINEAPPLES

CODEX STAN 184-1993 CODEX STANDARD FOR MANGOES

CODEX STAN 205-1997 CODEX STANDARD FOR BANANAS

CODEX STAN 219-1999 CODEX STANDARD FOR GRAPEFRUITS

CODEX STAN 245-2004 CODEX STANDARD FOR ORANGES

CODEX STAN 255-2007 CODEX STANDARD FOR TABLE GRAPES

**Team work "Development of a draft standard for a certain food product"**

*Objectives of Team work activity are:*

- to know a standard structure;
- to play a role of the standard developer and a reviewer.

*Steps of interactive technology* of team work activity includes the following stages:

*The first step* covers Information phase. Participants are given an introductory information about team work, its rules and tasks are stated (Duration is 15 minutes).

*Students are supposed to*

- Consider the type of fruit chosen (the list of products relevant to each student background will be provided). What are the main characteristics of this product?
- What clause and sub-clauses will you include in your draft standard for this product? Why?
- Develop content for each clause and sub-clause of your draft standard.
- Send the draft to major suppliers and retailers (your fellow-students) for reviewing.
- On your turn play the role of your fellow student draft standard reviewer. Provide remarks and comments on behalf of supplier and/or retailer.
- Consider the comments arrived. Will you agree with them? Why? Why not? Provide reasons.
- Present the final version of your standard.

*The second step* covers Organizational phase. Participants are divided into two teams: Developers and Reviewers of a standard given. After completing the task team players exchange their roles.

*The third step* covers Interactive phase. It includes the work on developing standards as well a review processing (Duration 40 minutes).

*The fourth step* covers the Closing remarks phase. the comments from the leaders and participants are given in Closing remarks.

*Participants in interactive technologies*

- Postgraduate students in the role of team players (standard developers and reviewers) — 4–8 persons.
- Leaders (lecturer and tutor) access team work of developers and reviewers — 1–2 people.

**Discussion points**

1. Define the abbreviations ISO, FAO, and WHO and give examples for each of what they do or regulate relevant to food analysis.
2. Differentiate "standards of identity", "standards of fill" and "grade standards" with regard to what kind of requirement they establish.
3. Upon completing your Master degree, you are employed by a major international food company that processes fruits and vegetables. Where, specifically, would you look to find if a standard of identity exists for each of your processed products?
4. You are considering marketing some of your products internationally. What resource could you check to determine if there are international standards and safety practices specified for those products?
5. What do you think the world would be like without standards?
6. In what way do standards influence people's life? Provide good examples.
7. What is a standard? Give the definition. What types of standards do you know?
8. What groups of people are involved in developing standards?
9. Do unique national standards have a future?

## Literature used

CODEX STAN 182-1993 CODEX STANDARD FOR PINEAPPLES // Codex Alimentarius Commission. Fresh Fruits and Vegetables, Food and Agriculture Organization of the United Nations (Food & Agriculture Org.). Business & Economics. Rome, Italy, 2007. C. 101–106.

CODEX STAN 184-1993 CODEX STANDARD FOR MANGOES // Codex Alimentarius Commission. Fresh Fruits and Vegetables, Food and Agriculture Organization of the United Nations (Food & Agriculture Org.). Business & Economics. Rome, Italy, 2007. C. 70–74.

CODEX STAN 205-1997 CODEX STANDARD FOR BANANAS // Codex Alimentarius Commission. Fresh Fruits and Vegetables, Food and Agriculture Organization of the United Nations (Food & Agriculture Org.). Business & Economics. Rome, Italy, 2007. C. 18–23.

CODEX STAN 219-1999 CODEX STANDARD FOR GRAPEFRUITS // Codex Alimentarius Commission. Fresh Fruits and Vegetables, Food and Agriculture Organization of the United Nations (Food & Agriculture Org.). Business & Economics. Rome, Italy, 2007. C. 44–49.

CODEX STAN 245-2004 CODEX STANDARD FOR ORANGES // Codex Alimentarius Commission. Fresh Fruits and Vegetables, Food and Agriculture Organization of the United Nations (Food & Agriculture Org.). Business & Economics. Rome, Italy, 2007. C. 89–95.

CODEX STAN 255-2007 CODEX STANDARD FOR TABLE GRAPES// Codex Alimentarius Commission. Fresh Fruits and Vegetables, Food and Agriculture Organization of the United Nations (Food & Agriculture Org.). Business & Economics. Rome, Italy, 2007. C. 134–138.

# Tutorial 2. Standard Sampling and Sample Preparation Procedures

### Basic Topics

1. Standard Sampling in Food Biotechnology.
2. Sample Standard Report and Sample Preparation Procedures.

## Tutorial Task. Documentation of Sampling and Sample Preparation

*Students are supposed to*

- Watch the video film featuring the process of cheese sample preparation (http://www.youtube.com/watch?v=IfPbla3ljKk).
- What are the major steps of the procedure applied? Give reasons (*Annex 2*).
- Provide a Sampling Report according to the following form (*Annex 3*).
- Are there any points you would like to include or exclude from this report? Why?

## Discussion Points

1. Identify at least three reasons you might need to determine certain chemical characteristics of a food product as part of a quality management program.
2. As part of your job as a supervisor in a quality assurance laboratory, you need to give a new employee an instruction regarding choosing a sampling plan. Which general factors would you discuss with the new employee? Distinguish between sampling for attributes vs. sampling for variables. Differentiate three basic sampling plans and the risks associated with selecting a plan.
3. What precautions should be taken to ensure that the sample composition is not changed during preparation?

## Literature used

ISO 707:2008 Milk and milk products. Guidance on sampling // European Committee for Standartization. EU. 2008. 40 p. URL: http://www.iso.org/iso/catalogue_detail.htm?csnumber=37882 (Accessed: 15 December 2015).

ISO 5538:2004 Milk and milk products. Sampling. Inspection by attributes // European Committee for Standartization. EU. 2004. 20 p. URL: http://www.iso.org/iso/catalogue_detail.htm?csnumber=37882 (Accessed: 15 December 2015).

ISO 8197:1988 Milk and milk products. Sampling. Inspection by variables// European Committee for Standartization. EU. 1988. 5 p. URL: http://

www.iso.org/iso/catalogue_detail.htm?csnumber=37882 (Accessed: 15 December 2015).

Codex Standard 234-1999 Recommended methods of analysis and sampling// Codex Alimentarius Commission. WHO. 1999. 63 p. URL: www.fao.org/input/download/standards/388/CXS_234e_2015.pdf. (Accessed: 15 December 2015).

## Tutorial 3. Food Labeling Regulation

**Basic Topics**

1. Nutritional Value as **the** Major Issue **of** Food Labeling.
2. The standard format for nutrition information.
3. Daily Values or Nutrient Reference Value.
4. Serving Size.
5. Rounding Rules for Declaring Nutrients on Nutrition Label.
6. Nutrient Content Claims.

**Tutorial Task. Study of the nutrition label enclosed.**

*Students are supposed to answer a tutor questions and justify their answers. They obtain 4 Labels of different foodstuffs before starting thinking*

**Questions**

- Can you make a "low fat" claim? Explain your answer.
- Could you use the term "healthy" on the label?

You may use any lecture notes at your choice.

**Discussion Points**

1. What is required to be on a food label?
2. What does "Daily Value" mean ?
3. What is a difference between Daily Reference Value (DRV) and Reference Daily Intake (DRI) ?
4. What kind of rules exist for rounding the numerical expression of quantity of all nutrients per serving ?
5. What is nutrient content claim ?

**Literature used**

DA Food Labeling Requirements E-book // foodpackaginglabels.net : portal. URL: http://www.foodpackaginglabels.net/food-labeling-requirements/ (Accessed: 15 January 2015).

Food Labeling Guide // fda.gov : portal. URL: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/LabelingNutrition/ucm2006828.htm (Accessed: 15 January 2015).

# Tutorial 4. Wine analysis. Types and characteristics of wines

**Basic Topics**

1. Main winery operations.
2. Harvest decisions.
3. Vineyard Sampling.
4. Berry Expansion During Ripening.
5. Sugar and Organic Acid during Ripening. Sugar measurement.
6. Ethanol Prediction.

**Quiz Show "How white and red wine has been made?"** *Objectives of Quiz Show* (*Team work activity*) *are*:

- to revise wine processing;
- to reveal the difference between red and white wine processing.

***Steps of interactive technology***

Quiz Show includes the following stages:

*The first stage* covers Information phase. Participants are given an introductory information about Quiz Show and its rules and assessment. (Duration is 15 minutes.)

*The second stage* covers Organizational phase. Participants are divided into two teams.

*The third stage* covers Interactive phase. It includes Quiz Show itself (Duration 35–40 minutes).

*The fourth stage* covers the Closing remarks phase. a winner of this quiz is identified. the comments from the leaders and participants are given in Closing remarks.

***Participants in interactive technologies***

- Postgraduate students in the role of team players — 4–8 persons.
- Leaders (lecturer and tutor) — 1–2 people.

**Discussion Points**

1. Briefly describe 5 stages of the wine brewing process.
2. Identify three stages of berry growth ripening.
3. How soluble solids and titratable acidity of juice from berries change with days after anthesis?
4. How to measure sugar, ethanol and organic acids content in wines?
5. What other parameters of wine should be measured as well?
6. What is the difference between manual and machine harvesting?
7. What key points the record of the recital of any wine good must contain?

**Literature used**

*Grainger K.* Wine Production: Vine to bottle / Keith and Hazel Tattersall. Food industry briefing series / K. Grainger. Wiley-Blackwell Publishing, 2005. 152 p.

*Tamang J. P.* Fermented Foods and Beverages of the World. Technology & Engineering / J. P. Tamang, K. Kailasapathy. CRC Press, Taylor and Francis Group, 2010. P. 85–126.

Wine Fermentation // Molecular Techniques in the Microbial Ecology of Fermented Foods / ed. by L. Cocolin, D. Ercolini. Springer, 2008. P. 162–192.

## Tutorial 5. Food Additives and Biologically Active Substances

**Basic Topics**

1. Terms and definitions.
2. Classification of food additives and biologically active additives.

3. The legislative and regulatory framework for food and biologically active additives.

**Tutorial Task 1. Name the food from each ingredient list below.**

1. _____Sugar, Sweet Dairy Whey, Com Syrup Solids, Cocoa Processed With Alkali, Partially, Hydrogenated Vegetable Oil (May contain one or more of the following oils: Corn, Canola or Sunflower), Nonfat Dry Milk, Cellulose Gum, Salt, Sodium Caseinate (A Milk Derivative), Artificial Vanilla Flavor.

2. _____Sugar, Corn Syrup, Chocolate (Chocolate Liquor Processed With Alkali, Sugar, Cocoa Butter, Chocolate Liquor, Cocoa, Clarified Butteroil and Soy Lecithin Added As An Emulsifier), Soya Protein, Salt, Peppermint Oil, Invertase.

3. _____ Dried Potatoes, Vegetable Oil (Contains One Or Cottonseed Oil, and/or Sunflower oil), Maltodextrin, Wheat Starch and Yellow corn Meal. Contains 2 % or Less of Sugar, Dextrose, Torula Yeast, Salt, Tomato Powder, Malted Barley Flour, Paprika (Color), Monosodium Glutamate, Onion Powder, Garlic Powder, Spices, Paprika Oleoresin (Color), Red Pepper, Citric Acid, and Natural Flavor.

4. _____ Enriched Bleached Flour (Wheat Flour, Niacin, Reduced Iron, Thiamin Mononitrate, Riboflavin, Folic Acid), Corn Syrup, Sugar, Vegetable Shortening (Partially Hydrogenated Soybean and Cottonseed Oils), Dextrose, Water, Cocoa, Walnuts, High Fructose Corn Syrup, Whey, Eggs, Soy Lecithin, Egg Whites, Salt, Leavening (Baking Soda and Sodium Aluminum Phosphate), Caramel Color, Corn Starch, Artificial Flavors, Red 40, Sorbic Acid (To Retain Freshness).

5. _____Sugar, Enriched Flour (Wheat Flour, Niacin, Reduced Iron, Thiamin, Mono-Nitrate, Riboflavin), Water, Egg Whites, Whole Eggs, Corn Syrup, Vegetable Shortening (Partially Hydrogenated Soybean Oil and/or Cottonseed Oil, Mono- and Diglycerides, Skim Milk, Macaroon Coconut (Processed with Sodium Bisulfite) Leavening (Soda, Sodium Acid Pyrophosphate,

Monocalcium Phosphate), Whey, Modified Food Starch, Salt, Gelatin, Soy Flour, Cocoa (Processed With Alkali), Natural and Artificial Flavor, Sodium Propionate and Sorbic Acid (Preservatives), Calcium Carbonate, Calcium Sulfate, Agar, Locus Bean Gum, Sodium Phosphate, Red № 3 and Red № 40.

6. _____ Cream, sugar, water, milk fat substitute, skim milk powder, milk protein, cocoa powder, emulsifier E452 and E339, acidity regulator — citric acid, preservative sorbic acid, semi-solid cheese, stabilizer carrageenan.

7. _____Non-fat Milk, Water, Sugar, Modified Food Starch, Maltodextrin, Salt, Carrageenan, Sodium Stearoyl Lactylate, Artificial and Natural Flavors, Color Added (Including Yellow 5 and 6).

8. _____ One set of ingredients — Salt, Monosodium Glutamate, Hydrolyzed Corn and Soy Protein, Dehydrated Vegetables (onions, Garlic), Sugar, Chicken Flavor (Hydrogenated Soybean Oil, Chicken Fat, Chicken Broth Solid), Spices, Chinese Cabbage Extract, Powdered Cooked Chicken, Natural Butter Flavor, Turmeric, Disodium Inosinate, Disodium Guanylate. Second set of ingredients — Enriched Wheat Flour (Wheat Flour, Niacin Reduced iron, Thiamin Mononitrate, Riboflavin), Partially Hydrogenated Vegetable Oil (Contains one or more of the following: Canola, Cottonseed, Palm), Salt, Soy Sauce (Water, Wheat, Soybeans, Salt), Potassium Carbonate, Sodium Phosphates, Sodium Carbonate, Turmeric.

9. _____Milk Chocolate (Sugar, Cocoa Butter, Skim Milk, Chocolate, Milkfat, Lactose, Soy Lecithin, Artificial Flavor), Corn Syrup, Sugar, Partially Hydrogenated Soybean Oil, Milk, Less Than 2 % — Cocoa Powder, Malted Barley, Lactose, Wheat Flour, Salt, Egg Whites, Soy Protein, Artificial Flavor.

10. _____ Low-fat cottage cheese, cream, skim milk, sugar, filler (sugar syrup, caramel syrup, glucose-fructose syrup, thickeners — E1442, E471, pectin, acidity regulators — citric acid, sodium citrate, caramelized sugar, flavor identical to natural one).

**Tutorial Task 2. Sample product for your choice are given below:**

Fig. 1. Creme filled Snow-ball[1]


Fig. 2. Hunt's Fat free Vanilla[2]



Fig. 3 Chicken flavored ramen noodles[3]


Fig. 4. Milky way chocolate sweet[4]

[1] A SNOW-BALL is a confection made with finely shaved ice and flavored cane sugar syrup (http://leitesculinaria.com/7520/ recipes-pink-snowballs-sno-balls. html#comments).

[2] https://www.soap.com/p/productname-316514.

[3] http://www.seriouseats.com/2013/09/taste-test-instant-ramen-chicken-flavored. html.

[4] https://www.buzzfeed.com/ailbhemalone/the-definitive-ranking-of-chocolate-bars-from-worst-to-best?utm_term=.enlknybkQX#.jeP4JGD4Py.


Fig. 5. Omichka
(Russian processed dessert)[6]


Fig. 6. Thin mint candy[5]


Fig. 7. Danissimo Danone
(curd product)[7]

Fig. 8. Miss Debbie
fudge Brownie[8]

[5] http://theconsumerscorner.net/thin-mint-girl-scouts-candy-bar/.

[6] http://irecommend.ru/content/syr-bez-syra-ili-nash-otvet-zamorskim-sladostyam-razbor-sostava.

[7] https://av.ru/i/192114/.

[8] http://lifemadesimplebakes.com/2014/11/homemade-little-debbie-fudge-brownies/.

Fig. 9. Miss Debbie fudge Brownie[9]


Fig. 10. Carnation hot cocoa mix[10]

**Tutorial Task 3. Most packaged foods contain additives to enhance the taste, appearance, or shelf life of the product. For the following food additives (Table 1), identify its function in food products, foods it is found in, and any problems associated with its use.**

*Table 1*

**Food additives, their function, food sources and problems related to them**

| Additive | Function | Food Sources | Problems (if any) |
|---|---|---|---|
| Aspartame | | | |
| Butylated Hydroxyanisole (BHA) | | | |
| Butylated Hydroxytoluene (BHT) | | | |
| Sodium Chloride | | | |
| Tartaric acid | | | |
| Sodium Benzoate | | | |
| Saccharin | | | |
| Fructose | | | |

[9] https://en.wikipedia.org/wiki/Pringles#/media/File:Pringles_chips.JPG.

[10] https://shop.freshstmarket.com/shop/product/nestle-carnation-hot-chocolate-mix-marshmallow/.

**Discussion Points**

1. What are food additives? What is their fundamental difference from the biologically active additives to food?
2. Justify the need of their application in technology in food stuff?
3. Can food additives be dangerous to humans?
4. the most important condition for ensuring the safety of foods with food additives.
5. Groups and functional classes of food additives.
6. Interpretation of the particular substance or group of substances food additive status.
7. What document regulates the use of food additives?
8. Control of the use of food additives.
9. Sanitary-hygienic examination of food additives.
10. Dietary supplements and supplements as a means of prevention and treatment of dangerous diseases.
11. Harmful and banned supplements in Russia.

**Literature used**

*Branen L. A.* Food Additives / L. A. Branen M. P. Davidson, S. Salminen, J. Thorngate. CRC Press (Technology & Engineering), 2001. 952 p.

Food Additives Data Book / ed. by Jim Smith, Lily Hong-Shum. Wiley-Blackwell, 2011. 1110 p.

Food Science Experiment and Activity Guide / Home Economics Education, North Carolina Department of Public Instruction, Raleigh, North Carolina, 1994. P. 49–62.

## Tutorial 6. Methods for detection and analysis of dioxines, other polyhalogenated hydrocarbons, hormonal drugs, antibiotics and micotoxins in food stuff and raw materials

**Basic Topics**

1. Dioxines, polychlorinated biphenyls and other polyhalogenated hydrocarbons as xenobiotics of food products.
2. Methods of analysis of polyhalogenated hydrocarbons in food stuff and environmental objects.

3. The problems of application and control of hormonal drugs and antibiotics in food raw materials, food raw materials.
4. Contamination of and food stuff with micotoxines and methods for their determination.

**Discussion Points**

1. Characterize toxic effect of polycyclic aromatic hydrocarbons?
2. What kind of substance is used as an indicator of polycyclic aromatic hydrocarbons in foodstuffs?
3. Specify the methods of benz(a)pyrene determination?
4. What kind of principles are fundamentals of different types of hormones classification and substances with hormonal activity?
5. Characterize potential danger of antibiotics in food stuffs.
6. Specify the methods for determination of residual amounts of antibiotics.
7. Characterize micotoxins of different groups in depending on the sources of penetration into food stuffs and their toxic effect on human and animals organisms.

**Literature used**

*Richardson M.* Environmental Xenobiotics / M. Richardson. CRC Press, 1996. 492 p.

*Püssa T.* Principles of Food Toxicology, Second Edition / T. Püssa. CRC Press, 1996. 492 p.

## Tutorial 7. Quantitative and qualitative determination of genetically modified sources (GMS) of plant origin in foodstuff

**Basic Topics**

1. Methods for quantitative and qualitative determination of the genetically modified sources (GMS) of plant origin in food stuff.
2. The method for chain reaction (PCR).
3. The PCR-laboratory's setup in food biotechnological enterprise.

**Homework Questions**

1. List the major steps that constitute the essence of the chain reaction method.
2. What is the purpose of genetic modification of agricultural plants and animals?
3. What kind of regulatory documents establish methods identify products from GMS?

**Tutorial Task 1. Discuss How Can Genetic Engineering Benefit Us in Use Today and Projected For the Future. Students should provide a tutor with their ideas by sequentially filling Table 2.**

*Table 2*

**Sampling of Present and Future Benefits from Genetic Engineering**

| Application | Benefit | In Use Today | Projected for the Future |
|---|---|---|---|
| Medicine | | | |
| Industry | | | |
| Energy | | | |
| Environment | | | |
| Agriculture | | | |

**Tutorial Task 2. Discuss Benefits and Negative Effects of Genetically Modified Food by sequentially filling Table 3.**

*Table 3*

**Benefits and Negative Effects of Genetically Modified Food**

| Benefit | Negative Effects |
|---|---|
| | |
| | |
| | |
| | |

Make short final conclusion om prospects of genetically modified food.

**Tutorial Task 3.** In class, discuss or debate ethical or moral issues associated with the advances in genetic engineering and other biotechnologies as follows:

- Divide the class into groups of three or four students.
- Each group will be presented with several reports of the ethical issues involved with the various advances being made in biotechnology.
- Have each group make a list of the important points raised by the articles that they reviewed.
- Within each group, debate the ethical issues raised by the articles.

*Make a short final conclusion om prospects of genetically modified food.*

**Literature used**

Genetically Modified Food Sources, Safety Assessment and Control / ed. by V. Tutelyan. 1st Edition. Academic Press, 2013. 368 p.

Food Science Experiment and Activity Guide / Home Economics Education, North Carolina Department of Public Instruction, Raleigh, North Carolina, 1994. P. 95–103.

## Tutorial 8. Food Irradiation

**Basic Topics**

1. Natural and artificial sources of exposures.
2. the basic principles for radioactive protection nutrition.
3. Legal and regulatory framework of radiation safety.

**Tutorial Task**

Foods are irradiated to destroy insects, food-spoiling and disease-causing bacteria and to lengthen the storage time for foods in warehouses and homes. Hospitals may use irradiation to sterilize foods for immuno-compromised patients. Irradiated foods such as strawberries, onions, and poultry are available to consumers. Although irradiated foods cannot be recognized by sight, smell, taste or feel, they will be

labeled with an irradiation logo plus the words "*Treated with Radiation*" or "*Treated by Irradiation*".

This tutorial explores whether or not irradiation makes a difference in appearance or taste. As fresh as stored samples of food irradiated and non-irradiated should be submitted to audience. the results of observation and testing should be placed in Table 4.

*Procedure:*

1. Obtain food samples; label either as **irradiated** or **not irradiated**.
2. Examine the appearance of each sample. Record differences in appearance.
3. Taste each sample. Record differences in taste.
4. Store food samples according to package directions or as typically recommended, depending on the food sample.
5. Observe and record changes in the stored foods on a regular basis, depending on the food samples. Length of observation times will vary with types of food samples. *Strawberries will deteriorate rapidly; onions or potatoes may take weeks for deterioration to begin.*

**Discussion Points**

1. Define the term radioactivity. What units of measurement of radioactivity?
2. What the most hazardous technogenic radionuclides can be found in food stuff?
3. What is biological action of radiation?
4. How radionuclides are spread in human body and in the bodies of agricultural animals?
5. Specify the basic principles of radiation protective nutrition.
6. Characterize International regulations related to radiation safety.
7. Does irradiation make food radioactive?
8. Does eating irradiated food present long-term health risks?
9. Will risks of radiation exposure increase significantly if you live next to an irradiator?

*Table 4*

**Comparison of Irradiated and Non-Irradiated Food**

| Food | Observations | | | |
|---|---|---|---|---|
| | Appearance | Taste | Stored Observations | Irradiation Yes or No |
| | | | 1st | |
| Strawberries | | | 2nd | |
| | | | 3rd | |
| | | | 1st | |
| Apple | | | 2nd | |
| | | | 3rd | |
| | | | 1st | |
| Potato | | | 2nd | |
| | | | 3rd | |
| | | | 1st | |
| Onion | | | 2nd | |
| | | | 3rd | |
| | | | 1st | |
| Other | | | 2nd | |
| | | | 3rd | |

*Related Questions and Recommendations*

1. Does irradiation alter the appearance and/or taste of the food you have tested?
2. Make personal recommendation for or against irradiation based on outside reading. Justify your response.

## Literature used

General Standard For Irradiated Foods CODEX STAN 106-1983, REV.1-2003 // Codex Alimentarius Commission. URL: ftp://ftp.fao.org/Codex/Meetings/CCFAC/ccfac33/fa01_12e.pd (Accessed: 15 January, 2015).

Food Irradiation: Principles and Applications / ed. by R. A. Molins. Wiley, 2001. 488 p.
Food Science Experiment and Activity Guide / Home Economics Education, North Carolina Department of Public Instruction, Raleigh, North Carolina, 1994. P. 63–72.

## Tutorial 9. Food Packaging Technology

### Backgrounds

Methods of packaging foods continually change as technological advancements are made and as needs and wants are expressed by consumers. Consider *aseptic packaging*. Juice boxes are an example. to slow down bacterial growth, the atmosphere is modified within the package with the addition of a mixture of $CO_2$, $O_2$ and $N_2$. This example illustrates how consumer demands impact technological advancements and developments in packaging. Packaging materials are subjected to extensive testing before they are released to the American public. These materials have to meet standards that will hold up to the typical "use and abuse" within the processing plants as well as American homes. Listed below are examples of tests used on various types of packaging materials.

*Packing Material Tests*

*Bursting Strength* — to see how much pressure will cause the package to burst.

*Compression Strength* — to determine how many items can be stacked before damage occurs.

*Impact Strength* — to determine what happens to a package when it is dropped.

*Penetration of Fats* — to determine if fats moves across barriers.

*Seal Integrity Tests* — to determine if packaging materials hold printed inks and do not bleed, fade or rub off.

*Stiffness Test* — to determine how much force is needed to push an object that will not yield.

*Tear Force* — to determine force necessary to pull a package apart.

*Tensile Strength* — to determine force required to pull materials apart.

*Transmission of Water* — to determine if the material allows the migration of water as well as gas across barriers.

*Vacuum Testing* — to determine if seals holds or if they are defective.

**Basic Topics**

1. Types of Packaging Food Materials.
2. Hygiene examination of polymer and other materials in contact with food products.

**Tutorial Task. Testing as an aspect of packaging material by interactive technology**

Interactive technology is research method which objective is — to learn how to perform your own experiment to test a minimum of one aspect of packaging material (see above).

*Steps of interactive technology*

Technology of Research method consists of performance of the experiment after the written experiment has been approved by your lecturer. Each student should submit his/her findings to the class using the following format for his/her experiment. He or she is advised to:

1. State the Problem. *Describe the problem you want to solve; the statement may be written as a question.*
2. Gather Information. *Examine known information; use it to help to form hypothesis.*
3. Form a Hypothesis. *Prediction of what you think will happen.*
4. Collect Data through Experimentation
   - Test one variable at a time.
   - Compare variable to the control.
   - Record observations carefully.
   - Calculate data accurately.
5. Analyze Data *Examine results of experiment; do they agree with your hypothesis?*
6. Form a Conclusion. *Summarize your results and explain them.*

Items listed below include the minimum requirements and the scoring scale for your experiment design project.

1. Written Report of Experiment:
   - Problem stated.
   - Hypothesis formed.
   - Components organized using scientific method.
   - Data, calculations, and conclusions.
   - Well-organized; proper use of language conventions and composing characteristics.
2. Experiment Performance:
   - Execution of experiment
   - Condition of lab station and equipment
   - Breakage (if any)
   - Conduct

*Participants in interactive technologies*:

- Postgraduate students in the role of team players — 4–8 persons.
- Leaders (lecturer and tutor) — 1–2 people.

**Discussion Points**

1. Why polymeric and other packaging materials used in food industry, catering and market can be the sources of contaminating food stuff with foreign chemical substances ?
2. Specify the basic groups of toxicants originated from polymeric and other materials, which are probable contaminants of food stuffs.
3. How the polymeric and related materials safety monitoring is realized?

## Literature used

Food Packaging / ed. by T. Kadoya. Elsevier Inc., 1990. 424 p.

Food Science Experiment and Activity Guide / Home Economics Education, North Carolina Department of Public Instruction, Raleigh, North Carolina, 1994. P. 73–76.

Uncloaking the GFSI Requirements for Food Packaging / AIB Update JANUARY/FEBRUARY 2014. P. 9–11. URL: http//ibonline.org/aibonline_/www.aibonline.org/newsletter/Magazine/Jan_Feb2014/Uncloaking.pdf (Accessed: 15 January 2015).

# Chapter 2
# LABORATORY EXPERIMENTS

## Laboratory experiment 1. Quantitative determination of the antioxidant activity of biotechnology products and biologically active substances by EPR

### Backgrounds

An *antioxidant* is a molecule that inhibits the oxidation of other molecules. Antioxidants (preservatives) are natural or synthetic compounds. the well-known antioxidants are ascorbic acid (vitamin C), vitamin E, ß-carotene (vitamin A) and lycopene (constituent of tomatoes). They also include polyphenols: flavine and flavonoids (often found in vegetables), tannins (cocoa, coffee, tea), anthocyanins (red berries).

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. in turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.

Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress is damaging to cell structure and cell function by overly reactive oxygen-containing molecules and chronic excessive inflammation. Oxidative stress seems to play a significant role in many human diseases, including cancers. the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. For these reasons, oxidative stress

can be considered to be both the cause and the consequence of some diseases.

Food and drinks based on vegetable raw materials can serve as the sources of antioxidant substances for human being due to the antioxidant properties of biologically active substances such as phenolic compounds, vitamins, proteins, sugars, carboxylic and amino acids. Therefore, *antioxidant activity* (*AOA*) *of food is* one of the indicators that determine their *biological value.* Antioxidants are also commonly used to prevent oxidative deterioration of fats and fat-containing products during production and storage. However, the use of synthetic antioxidants is limited due to their potential toxic effects. It leads to the need to find the alternative compounds in plant material with high antioxidant activity and which are harmless to humans[1].

*Beer and wine* are two of the most popular alcoholic beverages. They contain organic acids and a source of vitamins B1, PP (beer and wine), B2 and B12 (beer). in comparison with other alcoholic beverages, they have a fairly high nutritional value due to the content of potassium, sodium, calcium, phosphorus and iron (only red wine). Both beverages contain large amounts of phenolic compounds (1,5–4 g/dm$^3$), which cause their antioxidant activity. the source of phenolic compounds in beer are grain and malt. Also the antioxidant properties of beer are affected by sulfites.

*Juice*, especially fresh one, is a rich source of vitamin C and E and, in some cases, provitamin a (carrot juice). They show the highest antioxidant activity.

*Biologically active substances* with antioxidant properties, typically compositions, are natural and synthetic antioxidants with enhanced antioxidant activity in comparison with food products.

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[1] *Polak J., Bartoszek M., Stanimirova I.* A study of the antioxidant properties of beers using electron paramagnetic resonance // Food Chemistry. 2013. Vol. 141. P. 3042–3049 ; Magnetic Resonance in Food Science : An Exciting Future / ed. by J.-P. Renou, G. A. Webb, Peter S. Belton. RSC Publishing. 2010. 212 p.

*EPR spectroscopy in the experiments on antioxidant activity determination*

Spectroscopy of electron paramagnetic resonance (EPR) has been traditionally using for monitoring radiation processed foods for many years. Recently, this method has attracted attention in connection with research activities of antioxidants, as well as the study of the properties of dairy products, beer, wine, tea and coffee. EPR method is a direct method for determining the active free radicals which have been mentioned above as well as other paramagnetic particles.

The antioxidants molecules are not radicals and therefore can not be determined by EPR. However, these compounds can react fast enough with the active free radicals and other radicals, "quenching" them and thereby reducing their destructive effect on the body[2].

The activity of the antioxidant substances can be found exploring the reaction of foods containing antioxidants with stable radicals such as 1,1-diphenyl-2,2-picrylhydrazyl (DPPH) TEMPON, TEMPOL et al. Such type of reactions allows quantitatively determine effectiveness of their inhibiting by the decrease in the intensity of the EPR signal after a lapse of time since their inception.

*Objective of the work*

To determine the antioxidant activity of samples of wine, juice, beer and biological active food additives by ESR of stable radical 1,1-diphenyl-2,2-picrylhydrazyl (DPPH).

**Experimental**

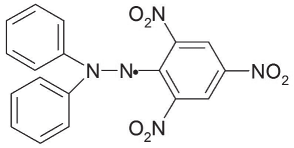
*Equipment, Reagents and Materials*

- EPR spectrometer Bruker Elexys E-500, X band (9.2–9.3 GHz);
- Radical DPPH solution in alcohol (c = 1 mM);
- Ascorbic acid solutions with c = 0, 0.125, 0.25 and 0.5 mM (used for plotting a calibration curve);
- Samples of wine, beer, dietary supplements — Anti-Oxidants;
- Capillaries;
- Distilled water.

[2] *Rohn S., Kroh L.* W. Electron spin resonance — a spectroscopic method for determining the antioxidative activity // Mol Nutr Food Res. 2005. Vol. 49. № 10. P. 898–907.

*Principle of determination of the antioxidant activity*

The stable radical DPPH has a formula (1):




(1)

DPPH is a common abbreviation for an organic 2,2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical molecules. DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidants assay, and another is a standard of the position and intensity of electron paramagnetic resonance signals.

DPPH serves as a reference both in solid state and in the liquid state when dissolved in organic solvent. the line width measured from the solid is subject to exchange narrowing and thus, varies from under 1 gauss to over 4 Gauss, depending on the solvent that was used for recrystallization. It has a ***g*** facëtor of 2.0036 ±0.0003. When dissolved in solution, a quintet with unresolved hyperfine couplings is observed because the spin exchange narrowing is reduced as the sample is diluted (Fig. 11).

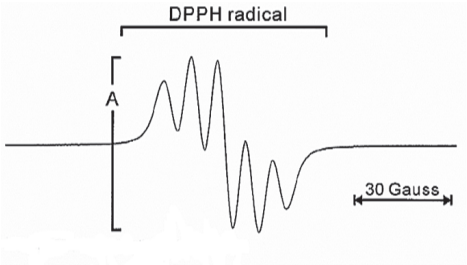



Fig. 11. EPR signal for solution with 100μM DPPH in methanol (1mT = 10 G), a is an intensity of the third component

The compounds with antioxidant activity will effect on the time and rate of the redox reaction with the DPPH, leading to a decrease in radical concentration in the reaction mixture and thus to a decrease in the EPR signal in time. the EPR signal of DPPH in water-alcohol mixture is shown in Fig. 12. Double integral of the DPPH EPR signal is proportional to the radical concentration and is used as the parameter to determine percentage of inhibition of the EPR spectrum of the radical in a mixture with a food sample studied.

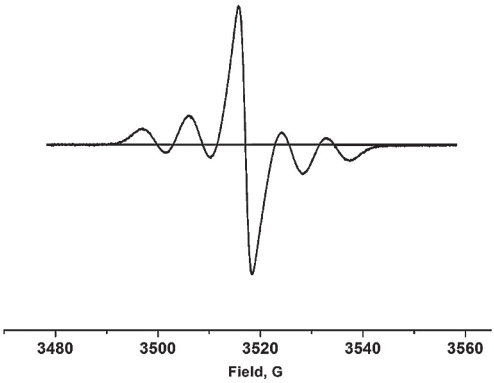



Fig. 12. EPR spectrum of DPPH in water-alcohol mixture

The antioxidant activity of food samples is determined in *mmol of Ascorbic acid per 1 liter of a sample.* the intensity of the EPR spectrum decreases with an increasing concentration of ascorbic acid, which results in an increase of the percentage inhibition (***I, %***). the percentage inhibition of the EPR spectrum was calculated using the following equation[3]:

$$I(\%) = \left[ \frac{I_0 - I}{I_0} \right] \times 100, \qquad (2)$$

[3] *Rohn S., Kroh L. W.* Electron spin resonance — a spectroscopic method… P. 898–907.

where $I_0$ is Double Integral of the EPR signal of water-ethanol mixture radical (500 µl of DPPH in ethanol: water 500 µl), $I$ — Double integral of the EPR signal of a mixture of DPPH in ethanol (500µl) and 500 µl of the sample. The EPR spectrum of DPPH changing in time in the process of reaction with an antioxidant is recorded three times after 3, 5 and 7 minutes from the start of the reaction. Double integral of DPPH signal is measured after 7 minutes from the start of the reaction.

To plot a calibration curve, the percentage of inhibition of the double integral of EPR signal of radical-ascorbic acid ($I$, %) should be presented as a function of ascorbic acid concentration in mM ($c_{Asc}$). Antioxidant activity of any studied sample is measured in *mmol of Ascorbic acid per 1 liter of a sample* using the calibration curve "*Percentage of inhibition of double integral EPR signal,* $I$ (%) *vs. ascorbic acid content,* $c_{Asc}$, *mM*".

*Laboratory guidelines:*

1. To plot the calibration curve 'Percentage of inhibition of double integral EPR signal, $I$ (%) vs. ascorbic acid content, $c_{Asc}$, mM'.
2. To measure antioxidant activity of the samples studied in *mmol of Ascorbic acid per 1 liter of a sample* using the calibration curve.
3. To compare the antioxidant activity of wine, beer, and dietary supplements and to make a conclusion which product shows the highest antioxidant activity and why.

**Questions**

1. Define antioxidant activity. Which natural products possess antioxidant activity ?
2. Review EPR based techniques of determination of antioxidant activity of food stuffs.
3. Describe the principle of determination of antioxidant activity based on application of free stable radical like DPPH.

# Laboratory Experiment 2. Quality control of milk and dairy products based on organoleptic and physico-chemical characteristics

## Backgrounds

National fermented milk product (NFMP) is a fermented milk product that has historically established name in Russia, which depends on the kind of ferment and specific technologies:

*Kefir* is a NFMP of mixed lactic acid and alcoholic fermentation produced by fermentation of sourdough prepared on kefir fungi without adding pure cultures of lactic acid bacteria and yeast. the lactic acid content of microorganisms in the final product at the end of expiry date is not less than $10^7$ CFU (Colony Forming Units) per 1 g of the product, and yeast at least $10^4$ CFU per 1 g of the product without the adding non-dairy components.

*Sour-milk* (*clabber*) is a NFMP produced by fermentation of milk with pure cultures of lactococci and/or thermophilic lactic streptococci. Their total content in the final product at the end of expiry date is not less than $10^7$ CFU per 1 g of the product, without adding non-dairy ingredients.

*Fermented baked milk* is a NFMP produced by fermentation of baked milk with pure cultures of thermophilic lactic streptococci. Their total content in the final product at the end of the expiry date is not less than $10^7$ CFU per 1 g of the product, without adding non-dairy ingredients.

*Butter from cow's milk* is a dairy product, which is the predominant part of milk fat manufactured only from cow's milk and/or products obtained from milk by isolating the fat phase and uniform distribution of milk plasma therein. *Butter* is ***a*** butter from cow's milk with a fat mass fraction from 50.0 % up to 85.0 %, representing the dispersion of "water in fat".

## Experiment 1. Determination of the organoleptic (sensory) characteristics of cultured milk products

Sensory evaluation of yogurt, acidophilus milk, kefir and koumiss consists in determining their *appearance, consistency, colour, taste and smell.*

According to the National Standards of the Russian Federation (GOST R 52093-2003, GOST 52094-2003, GOST 52095-2003)[4], organoleptic characteristics of the product must meet the requirements of Table 5.

*Table 5*

**Organoleptic characteristics of cultured milk products**

| Characteristic | Cultured Milk Product | | |
|---|---|---|---|
| | Kefir | Fermented Baked Milk | Clabber |
| Taste and smell | Pure, fermented without foreign tastes and odors. the taste is slightly spicy, allowed yeast flavor | Pure, fermented, with a pronounced taste of pasteurization, without foreign tastes and odors | Pure, fermented without foreign tastes and odors |
| Colour | Milky white, uniform throughout the mass | Light creamy, uniform throughout the mass | Milky white, uniform throughout the mass |
| Appearance and Consistency | Homogeneous with impaired or not impaired clot. Aerogenesis (gas formation) caused by the microflora of kefir fungi is allowed | Homogeneous with impaired or not impaired clot without gas formation | Homogeneous with impaired or not impaired clot |

[4] GOST R 52093-2003. Kefir. Technical Requirements. Implemented on 30 June, 2003. Moscow : Standards Publishing, 2003. 11 p. ; GOST 52094-2003. Fermented Baked Milk. Technical Requirements. Implemented on 30 June, 2003. Moscow : Standards Publishing, 2003. 11 p. ; GOST 52095-2003. Clabber (Sour Milk). Technical Requirements. Implemented on 30 June, 2003. Moscow : Standards Publishing, 2003. 11 p.

*Appearance and consistency.* Evaluation of appearance and quality of cultured milk products is carried out in the same manner as for milk assessment. When evaluating the appearance and consistency of kefir, it is necessary to pay attention to its homogeneity, the presence of sediment, floating clots and defend cream. the consistency of the product is provided with the nature of the clot generation method, the intensity of biochemical processes during manufacture and storage of products.

The products developed by thermostatic method are dense undisturbed clot as compared to those done by reservoir method. the latter have a disturbed clot of sour cream consistency.

In kefir, mare, fermented mare's and acidophilus milk clot is full of gas bubbles formed as a result of the activity of gaseous microorganisms and yeast made with sourdough. Gassing occurs in separate bubbles.

Consistency of dietary products of mixed fermentation is also determined while filling glasses how the product flows into the glass.

Clabber clot should be intact, undisturbed, without gassing. a sample taken with spoon should keep stable forms, clot kink should be lustrous. the serum concentration in clabber is required to be not more than 3 % by volume, in kefir — less than 2 %.

*Colour.* It is determined in the same manner as in milk. Milk is poured into a transparent glass and is examined in diffused daylight, paying attention to the presence of foreign shades.

*Taste and smell.* When determining taste and smell, it is necessary to pay attention to the purity of the cultured milk taste and the absence of other flavors. to determine taste it is required to take about 10 ml of a milk cultured product, to rinse mouth with it till the root of a tongue and to feel the presence of deviations from normal taste according to the GOST R.

*In the laboratory practice* it is proposed to compare organoleptic characteristics of kefir, fermented baked milk and clabber produced in accordance with all the requirements of GOST R 52093-2003 as well as the kefir sample subjected to damage.

The results of organoleptic should be collected in Table 6.

*Table 6*

**Results of organoleptic assessment**

| Quality characteristics | Requirements according to GOST 52093-2003 R | Quality of fresh product (fermented baked milk and clabber) | Quality of the kefir sample subjected to damage |
|---|---|---|---|
| Appearance and consistency<br>Colour<br>Smell<br>Taste | (should be taken from Table 5) | | |

## Experiment 2. Determination of physical and chemical parameters of milk and dairy products

Milk is normalized by mass fraction of fat, protein, titratable acidity, density, purity group, SNF (dry skimmed milk residue content) and temperature on leaving enterprise (GOST 52054, GOSTR 52791). For certain types of products, these characteristics are given in Table 7.

To quantify the physic-chemical characteristics of the selected sample of dairy products and fermented milk products (kefir) the following studies take place:

- determination of the temperature of the product on leaving enterprise and the amount of product produced according to GOST 3622;
- determination of the mass concentration of fat according to GOST 5867-90;
- determination of protein content according to GOST 23327;
- determination of acidity according to GOST 3624-92;
- determination of phosphatase in the product according to GOST 3623.

*Table 7*

**Physico-chemical characteristics of drinking milk**

| Characteristic | Mass concentration of fat in a product, %, not less than | | | | | |
|---|---|---|---|---|---|---|
| | For drinking milk | | | | | For milky drink |
| | skimmed, less than 0.5 | 0.5; 1.0 | 1.2; 1.5; 2.0; 2.5 | 2.7; 2.8; 3.0; 3.2; 3.5; 4.0; 4.5 | 4.7; 5.0; 5.5; 6.0; 6.5; 7.0; 7.2; 7.5; 8.0; 8.5; 8.9 | 0.5; 1.0; 1.2; 1.5; 2.0; 2.5; 2.7; 2.8 3.0; 3.2; 3.5; 4.0; 4.5; 4.7; 5.0; 5.5; 6.0 |
| Density, kg/m$^3$ not less than | 1030 | 1029 | 1028 | 1027 | 1024 | — |
| Mass concentration of protein, %, not less than | 2.8 | | | | | 2.2 |
| Acidity, °T, not more than | 21 | | | | 20 | 21 |
| Dry Skimmed milk residue content, % not more than | 8.2 | | | | | 7.0 |
| The temperature of the product on leaving enterprise, in °C: for pasteurized and baked milk; for ultra pasteurized and sterilized milk | 4 ± 2<br>from 2 up to 25 | | | | | |
| Group purity, not lower than | 1 | | | | | |

Physic-chemical characteristics of kefir, fermented baked milk, yogurt, clabber must satisfy the standards specified in Table 8.

*Table 8*

**Physico-chemical characteristics of kefir, fermented baked milk and clabber**

| Characteristic | Standard requirements | | | | | |
|---|---|---|---|---|---|---|
| | Skimmed | Non-fatty | Low-fat | Classi-cal | Fat | High-fatty |
| Mass concentration of protein, not less than, in % | 2.8 | | | 2.6 | | |
| Acidity, oT, not more than<br>Kefir<br>Fermented Baked Milk<br>Clabber | from 85 up to 130<br>from 70 up to 110<br>from 85 up to 130 | | | | | |
| The temperature of the product on leaving enterprise, about °C | 4 ± 2 | | | | | |

## Experiment 3. Determination of acidity of fresh dairy products (kefir, fermented baked milk and clabber ) and the kefir sample subjected to damage[5]

*Equipment, materials and reagents:*

- balances;
- thermometer with a measuring range of 0–100 °C and scale factor 0,1 °C;
- flasks, beakers, funnels, pipettes, cylinders, burettes;
- burette stand;
- 70 % alcohol solution of phenolphtaleine;
- 0.1 M solution of NaOH;
- Distilled water.

*Analysis.* 20 ml of distilled water, 10 ml of the sample product and 3 drops of phenolphthalein are added to 100–250 $cm^3$ flask. Analyzing a cultured milk product, its residue is transferred from the pipette

[5] GOST 3624-92. Milk and Diary Products. Titrimetric Methods of Acidity Determination. Technical Requirements. Implemented on 1 January, 1993. Moscow : Standards Publishing, 2001. 31 p.

(volumetric tube) into a conical flask washing the pipette (tube) used three times with the resulting mixture. Then the mixture is thoroughly stirred and titrated with sodium hydroxide solution until a slight pink coloration does not disappear within 1 minute.

*Data Processing.* Acidity in Turner degrees (°T) is determined by multiplying the volume of sodium hydroxide solution in $cm^3$ expended to neutralize acid contained in a certain volume of the product by a factor of 10. the difference between the parallel determinations should not exceed 2.6 °T. the final result of the analysis should be taken as the arithmetic mean of the results of two parallel determinations, rounding the result the second decimal.

In this practice it is proposed to compare acidity factors for 4 samples: Kefir, Kefir sample subjected to damage, Fermented Baked Milk and Clabber.

The results of determination should be collected in Table 9.

*Table 9*

**Results of determination of acidity of cultured milk products**

| Fermented product | Acidity, °T | Acidity according to GOST 3624-92 |
|---|---|---|
| Kefir | | |
| Kefir sample subjected to damage | | |
| Fermented Baked Milk | | |
| Clabber | | |

## Experiment 4. Determination of milk density[6]

*Density* (*bulk density*) is a mass at 20 °C in a unit of volume (g/ml). the density of natural cow milk lies in the range of 1.027–1.032 g/ml and depends on the temperature and composition of milk. the density

[6] GOST 3625-84. Milk and Dairy Products. Methods of Milk Density Determination. Technical Requirements. Implemented on 1 August, 1985. Moscow : Standards Publishing, 2009. 13 p.

increases as the amount of protein, carbohydrates, minerals increases and with reducing amounts of fat and water.

*Equipment.* Lactodensimeter of type a with a thermometer; glass cylinder of 250 ml.

*Analysis Procedure.* Milk density is determined not earlier than 2 hours after milking owing to the presence of suspended bubbles at 20 °C. to determine the density, well-mixed milk is poured into dry measuring cylinder of 250 or 500 ml, gently, along the wall to avoid the appearance of foam, then dry clean aerometer (lactodensimeter) is put into the milk and is left in a free-floating state. *It is necessary to make sure that it does not touch the sides and bottom of the cylinder!* After a minute, milk temperature can be measured using the upper scale of the lactodensimeter, whereas the bottom scale will show us the density if our eyes are set on the milk surface level (the upper edge of the meniscus). If the temperature is above or below the temperature of the milk (20 °C or below), the correction should be made for each degree of temperature difference by 0.0002 °C. This correction is added to the density found and is subtracted from this density at the milk temperature above and below 20 °C, respectively.

To bring the results of measurement to 20 °C the table from GOST 3625-84 can be used (Tables 10, 11), in which the density at the temperature of measurement is found in the left column, whereas in the head of the table the temperature at which the density was measured, is indicated. on the intersection of two columns the density of milk at 20 °C is determined.

*Example.* the density of milk 1.031 g/ml was measured at 15 °C. the difference in temperature was 20–15 °C = 5 °C; density correction was found equal to 5 °C × 0.0002 = 0.001 g/ml; therefore, the density of milk at 15 °C will be 1.031–0.001 = 1,030 g/ml. on the intersection of two columns (1030 kg/m$^3$) and 15 °C the density of milk at 20 °C was determined. It was equal to 1028.4 kg/m$^3$ or 1.0284 g/ml.

*Table 10*

**Table adjusting cow's milk density to 20 °C (Temperature range is from 15 up to 20 °C)**

| Measured density at certain temperature, kg/m³ | Milk density adjusting to 20 °C in kg/m³ at milk temperature, T, °C | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15.0 | 15.5 | 16.0 | 16.5 | 17.0 | 17.5 | 18.0 | 18.5 | 19.0 | 19.5 | 20 |
| 1025.0 | 1023.4 | 1023.6 | 1023.7 | 1023.9 | 1024.0 | 1024.2 | 1024.4 | 1024.5 | 1024.7 | 1024.8 | 1025.0 |
| 1025.5 | 1023.9 | 1024.1 | 1024.2 | 1024.4 | 1024.5 | 1024.7 | 1024.9 | 1025.0 | 1025.2 | 1025.3 | 1025.5 |
| 1026.0 | 1024.4 | 1024.6 | 1024.7 | 1024.9 | 1025.0 | 1025.2 | 1025.4 | 1025.5 | 1025.7 | 1025.8 | 1026.0 |
| 1026.5 | 1024.9 | 1025.1 | 1025.2 | 1025.4 | 1025.5 | 1025.7 | 1025.9 | 1026.0 | 1026.2 | 1026.3 | 1026.5 |
| 1027.0 | 1025.4 | 1025.6 | 1025.7 | 1025.9 | 1026.0 | 1026.2 | 1026.4 | 1026.5 | 1026.7 | 1026.8 | 1027.0 |
| 1027.5 | 1025.9 | 1026.1 | 1026.2 | 1026.4 | 1026.5 | 1026.7 | 1026.9 | 1027.0 | 1027.2 | 1027.3 | 1027.5 |
| 1028.0 | 1026.4 | 1026.6 | 1026.7 | 1026.9 | 1027.0 | 1027.2 | 1027.4 | 1027.5 | 1027.7 | 1027.8 | 1028.0 |
| 1028.5 | 1026.9 | 1027.1 | 1027.2 | 1027.4 | 1027.5 | 1027.7 | 1027.9 | 1028.0 | 1028.2 | 1028.3 | 1028.5 |
| 1029.0 | 1027.4 | 1027.6 | 1027.7 | 1027.9 | 1028.0 | 1028.2 | 1028.4 | 1028.5 | 1028.7 | 1028.8 | 1029.0 |
| 1029.5 | 1027.9 | 1028.1 | 1028.2 | 1028.4 | 1028.5 | 1028.7 | 1028.9 | 1029.0 | 1029.2 | 1029.3 | 1029.5 |
| 1030.0 | 1028.4 | 1028.6 | 1028.7 | 1028.9 | 1029.0 | 1029.2 | 1029.4 | 1029.5 | 1029.7 | 1029.8 | 1030.0 |
| 1030.5 | 1028.9 | 1029.1 | 1029.2 | 1029.4 | 1029.5 | 1029.7 | 1029.9 | 1030.0 | 1030.2 | 1030.3 | 1030.5 |
| 1031.0 | 1029.4 | 1029.6 | 1029.7 | 1029.9 | 1030.0 | 1030.2 | 1030.4 | 1030.5 | 1030.7 | 1030.8 | 1031.0 |
| 1031.5 | 1029.9 | 1030.1 | 1030.2 | 1030.4 | 1030.5 | 1030.7 | 1030.9 | 1031.0 | 1031.2 | 1031.3 | 1031.5 |
| 1032.0 | 1030.4 | 1030.6 | 1030.7 | 1030.9 | 1031.0 | 1031.2 | 1031.4 | 1031.5 | 1031.7 | 1031.8 | 1032.0 |
| 1032.5 | 1030.9 | 1031.1 | 1031.2 | 1031.4 | 1031.5 | 1031.7 | 1031.9 | 1032.0 | 1032.2 | 1032.3 | 1032.5 |
| 1033.0 | 1031.4 | 1031.6 | 1031.7 | 1031.9 | 1032.0 | 1032.2 | 1032.4 | 1032.5 | 1032.7 | 1032.8 | 1033.0 |
| 1033.5 | 1031.9 | 1032.1 | 1032.2 | 1032.4 | 1032.5 | 1032.7 | 1032.9 | 1033.0 | 1033.2 | 1033.3 | 1033.5 |
| 1034.0 | 1032.4 | 1032.6 | 1032.7 | 1032.9 | 1033.0 | 1033.2 | 1033.4 | 1033.5 | 1033.7 | 1033.8 | 1034.0 |
| 1034.5 | 1032.9 | 1033.1 | 1033.2 | 1033.4 | 1033.5 | 1033.7 | 1033.9 | 1034.0 | 1034.2 | 1034.3 | 1034.5 |
| 1035.0 | 1033.4 | 1033.6 | 1033.7 | 1033.9 | 1034.0 | 1034.2 | 1034.4 | 1034.5 | 1034.7 | 1034.8 | 1035.0 |
| 1035.5 | 1033.9 | 1034.1 | 1034.2 | 1034.4 | 1034.5 | 1034.7 | 1034.9 | 1035.0 | 1035.2 | 1035.3 | 1035.5 |
| 1036.0 | 1034.4 | 1034.6 | 1034.7 | 1034.9 | 1035.0 | 1035.2 | 1035.4 | 1035.5 | 1035.7 | 1035.8 | 1036.0 |

*Table 11*

**Table adjusting cow's milk density to 20 °C (Temperature range is from 20.5 up to 25.5 °C)**

| Measured density at certain temperature, kg/m³ | Milk density adjusting to 20 °C in kg/m³ at milk temperature, T, °C | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20.5 | 21.0 | 21.5 | 22.0 | 22.5 | 23.0 | 23.5 | 24.0 | 24.5 | 25.0 | 25.5 |
| 1025.0 | 1025.2 | 1025.3 | 1025.5 | 1025.6 | 1025.8 | 1026.0 | 1026.1 | 1024.5 | 1026.3 | 1026.4 | 1026.6 |
| 1025.5 | 1025.7 | 1025.8 | 1026.0 | 1026.1 | 1026.3 | 1026.5 | 1026.6 | 1025.0 | 1026.8 | 1026.9 | 1027.1 |
| 1026.0 | 1026.2 | 1026.3 | 1026.5 | 1026.6 | 1026.8 | 1027.0 | 1027.1 | 1025.5 | 1027.3 | 1027.4 | 1027.6 |
| 1026.5 | 1026.7 | 1026.8 | 1027.0 | 1027.1 | 1027.3 | 1027.5 | 1027.6 | 1026.0 | 1027.8 | 1027.9 | 1028.1 |
| 1027.0 | 1027.2 | 1027.3 | 1027.5 | 1027.6 | 1027.8 | 1028.0 | 1028.1 | 1026.5 | 1028.3 | 1028.4 | 1028.6 |
| 1027.5 | 1027.7 | 1027.8 | 1028.0 | 1028.1 | 1028.3 | 1028.5 | 1028.6 | 1027.0 | 1028.8 | 1028.9 | 1029.1 |
| 1028.0 | 1028.2 | 1028.3 | 1028.5 | 1028.6 | 1028.8 | 1029.0 | 1029.1 | 1027.5 | 1029.3 | 1029.4 | 1029.6 |
| 1028.5 | 1028.7 | 1028.8 | 1029.0 | 1029.1 | 1029.3 | 1029.5 | 1029.6 | 1028.0 | 1029.8 | 1029.9 | 1030.1 |
| 1029.0 | 1029.2 | 1029.3 | 1029.5 | 1029.6 | 1029.8 | 1030.0 | 1030.1 | 1028.5 | 1030.3 | 1030.4 | 1030.6 |
| 1029.5 | 1029.7 | 1029.8 | 1030.0 | 1030.1 | 1030.3 | 1030.5 | 1030.6 | 1029.0 | 1030.8 | 1030.9 | 1031.1 |
| 1030.0 | 1030.2 | 1030.3 | 1030.5 | 1030.6 | 1030.8 | 1031.0 | 1031.1 | 1029.5 | 1031.3 | 1031.4 | 1031.6 |
| 1030.5 | 1030.7 | 1030.8 | 1031.0 | 1031.1 | 1031.3 | 1031.5 | 1031.6 | 1030.0 | 1031.8 | 1031.9 | 1032.1 |
| 1031.0 | 1031.2 | 1031.3 | 1031.5 | 1031.6 | 1031.8 | 1032.0 | 1032.1 | 1030.5 | 1032.3 | 1032.4 | 1032.6 |
| 1031.5 | 1031.7 | 1031.8 | 1032.0 | 1032.1 | 1032.3 | 1032.5 | 1032.6 | 1031.0 | 1032.8 | 1032.9 | 1033.1 |
| 1032.0 | 1032.2 | 1032.3 | 1032.5 | 1032.6 | 1032.8 | 1033.0 | 1033.1 | 1031.5 | 1033.3 | 1033.4 | 1033.6 |
| 1032.5 | 1032.7 | 1032.8 | 1033.0 | 1033.1 | 1033.3 | 1033.5 | 1033.6 | 1032.0 | 1033.8 | 1033.9 | 1034.1 |
| 1033.0 | 1033.2 | 1033.3 | 1033.5 | 1033.6 | 1033.8 | 1034.0 | 1034.1 | 1032.5 | 1034.3 | 1034.4 | 1034.6 |
| 1033.5 | 1033.7 | 1033.8 | 1034.0 | 1034.1 | 1034.3 | 1034.5 | 1034.6 | 1033.0 | 1034.8 | 1034.9 | 1035.1 |
| 1034.0 | 1034.2 | 1034.3 | 1034.5 | 1034.6 | 1034.8 | 1035.0 | 1035.1 | 1033.5 | 1035.3 | 1035.4 | 1035.6 |
| 1034.5 | 1034.7 | 1034.8 | 1035.0 | 1035.1 | 1035.3 | 1035.5 | 1035.6 | 1034.0 | 1035.8 | 1035.9 | 1036.1 |
| 1035.0 | 1035.2 | 1035.3 | 1035.5 | 1035.6 | 1035.8 | 1036.0 | 1036.1 | 1034.5 | 1036.3 | 1036.4 | 1036.6 |
| 1035.5 | 1035.7 | 1035.8 | 1036.0 | 1036.1 | 1036.3 | 1036.5 | 1036.6 | 1035.0 | 1036.8 | 1036.9 | 1037.1 |
| 1036.0 | 1036.2 | 1036.3 | 1036.5 | 1036.6 | 1036.8 | 1037.0 | 1037.1 | 1035.5 | 1037.3 | 1037.4 | 1037.6 |

## Experiment 5. Determination of butter falsification

The simplest method for qualitative falsification of butter is achieved by reducing the content of milk fat. For example, "Amateur" oil might contain 76 and even 75 % of milk fat instead of 78 %, as provided by the current standard.

Adding of margarine or other hydrogenated fats into butter is defined by the following indicators:

1. The presence of antioxidants — butyloxitoluene (BHT), butylhydroxyanisole.
2. The reduced content of butyric acid.
3. The increased content of lauric acid.

*In this experiment, we can determine falsificated oil by several ways:*

*First method*

• Prepare the "explosive mixture" of alcohol and concentrated sulfuric acid in a ratio of 2:1. Then the melted butter is added to the mixture formed in the same ratio as well.

• Heat the mixture up to boiling and smell it after cooling down. If the cooled mixture has a pineapple smell, it is a real butter. If the mixture smells very unpleasant, it is margarine.

*Second method*

Put a small piece of oil into a tube, then heat it from the top so the oil goes down. Thereafter, the oil should be heated from the bottom up to boiling. If the oil is pure, it will turn black and begin release bubbles. And if it is margarine, it will brighten and boil rapid, spilling out.

**Questions**

1. What kinds of the Russian National Fermented Milk Products do you know? What is the difference between them?
2. What organoleptic characteristics had the kefir sample subjected to damage?
3. What are the standard requirements for physico-chemical characteristics of kefir?
4. How to measure the milk density? What kind of characteristics of milk effect on its density? And how?
5. Briefly describe the methods of qualitative falsification of butter from cow's milk.

# Laboratory Experiment 3. Determination of Caffeine in Coffee Products According to DIN 20481. Standard: DIN-DIN ISO 20481[7]

## Backgrounds

This International Standard specifies a high performance liquid chromatography (HPLC) method for the determination of the caffeine content of: green coffee; roasted coffee; soluble coffee, regular and decaffeinated; and mixed instant coffee products (e. g. coffee/chicory mix or cappuccino-type coffee drink).

Today, coffee is the second most valuable product (besides crude oil) exported from developing countries, with a trading volume of approximately 22 billion US $. in 2007, the world coffee production was approximately 7,742,675 tons, with Brasilia as the largest producer of approximately 28 %. on the consumer side, the USA has the highest total consumption and Finland the highest consumption per person. One of the main ingredients of coffee is the alkaloid caffeine, with approximately 80–120 mg per cup. Caffeine is responsible for the stimulating effect of coffee. the regular coffee product contains approximately 2–4 % of caffeine based on the used instant coffee granules.

Decaffeinated coffee is produced in large amounts by extraction of the caffeine from green coffee beans with hot water, organic solvents, or supercritical carbon dioxide. Decaffeinated coffee must contain less than 0.1 % caffeine.

In the European Union, only beverages that do not typically contain caffeine, for example energy drinks, must be labeled with the amount of caffeine the beverages contain.

The measurement of caffeine in coffee products is standardized in the DIN ISO regulations. Besides caffeine, other important

[7] Coffee and coffee products — Determination of the caffeine content using high performance liquid chromatography (HPLC) — Reference method (ISO 20481:2008) // ISO portal. URL: http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=34185 (Accessed: 15 January 2015)

compounds inherent in coffee have to be controlled like chlorogenic acids, 16-O-methyl cafestol and contaminants such as mycotoxins.

**Experimental**

*Equipment*

- Agilent 1200 Infinity LC System:
- Agilent 1200 with G1513D DAD (Diode Array Detector)
- Agilent 1200 Infinity Binary Pump (G1312B) with external degasser (G1322A)
- Agilent 1200 Infinity Standard Autosampler (G1329B) with Sample Thermostat (G1330B)
- Agilent 1200 Infinity Thermostatted Column Compartment (G1316A)

*Software*

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.05.

*Column*

Agilent Zorbax SB 18 2.1×150 3.5 um.

*Chemicals*

All Chemicals were purchased from Sigma/Aldrich, Germany. Acetonitryl Biossolve was used HPLS-S grade. Fresh millipore deionized water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22 pm membrane point-of-use cartridge (Millipak). Regular and instant coffee were purchased from a local super markets.

*Sample preparation*

A batch of 0.25 g of coffee powder should be mixed with 2.5 mg MgO and 100 ml of hot (90 °C) water should be added. Then a mixture should be stirred in a water bath at 90 °C with a magnetic stirrer for 20 minutes. After stirring the extract-containing solution should be cooled down up to room temperature and an aliquot of 10 ml should be taken. An aliquot should be filtered with a filter paper and using microcentrifuge Microspin and injected in HPLC.

*HPLC method*

HPLC method parameters are given in Table 12.

*Table 12*

**HPLC method parameters**

| Parameter | Value |
|---|---|
| Solvents | Water, 0,1 % formic acid; B) Acetonitrile |
| Flow rate | 0,35 ml/min |
| Elution conditions | Isocratic, 8 % Acetonitrile |
| Stop time | 12 minutes |
| Injection volume | 3 µl |
| Needle wash | In a vial with Acetonitrile |
| Column temperature | 25 °C |
| Detection | 280 nm, Bandwidth 4 nm, Reference 360 nm |

*Standards*

Caffeine stock solution: 1.2 mg caffeine (water free) was dissolved in 1 ml in a vial (98 % caffeine solution).

*Basics of standard addition method*

The standard additions method (often referred to as "spiking" the sample) is commonly used to determine the concentration of a standard that is in a complex matrix such as biological fluids, soil samples, food etc. the reason for using the standard additions method is that the matrix may contain other components that interfere with the standard signal causing inaccuracy in the determined concentration.

This method is most advantageous because it virtually removes any interference due to medium effect. the idea is to add standard to the sample ("spike" the sample) and monitor the change in instrument response. the change in instrument response between the sample and the spiked samples is assumed to be due only to change in standard concentration.

**HPLC Experiment and Caffeine concentration determination**

An aliquot of 10 μl of standard solution of caffeine was added to 997 μl of extract (native) solution. Then the chromatograms of the extract solution without standard addition (native) (1) and with standard addition (2) were recorded using UV-Visible and total ion (TIC) detectors. Qualitatively caffeine is detected by a signal at m/z = 195 on mass spectrum.

As it is known, the area under a specific chromatographic peak (double integral) is proportional to sample concentration. in the current experiment is required to find the area under the caffeine peak on the UV-Visible chromatogram.

Quantitative determination of the caffeine concentration in a test aliquot solution by standard addition method can be performed using the formula (3):

$$x = \frac{A \times a}{(B - A)}, \tag{3}$$

where $x$ is the caffeine concentration in a test aliquot, mg/ml; a and $B$ are the double integrals of the chromatographic peaks of caffeine in the test solution without standard addition and with standard addition in the test solution; a is the standard addition of caffeine, mg/ml.

The caffeine content in coffee powder (c, mg caffeine/g coffee powder) can be calculated by the formula (4):

$$c = \frac{x \times V}{m}, \tag{4}$$

where $V$ (100 ml) is the volume of the initial solution, in which 0.25 g of coffee powder (m, g) was added. Also caffeine content should be estimated in per cent.

Make a conclusion about quality of your coffee sample based on caffeine content.

**Questions**

1. What is approximate caffeine content for regular, instant and low-quality coffee?
2. Why was MgO used in the process of HPLC probe preparation?
3. What is the main advantage of the method of standard additions as compared to the graduation plot technique? Which objects are more preferable for the method of standard additions?

## Laboratory Experiment 4. Spectrophotometric Determination of Nitrites in Sausages

**Backgrounds**

Product quality is defined as a set of properties that determine its ability to meet specific needs in accordance with a purpose.

Meat and meat products are among the most valuable food. the components included in the meat are the starting material for the construction of tissues, biosynthesis of essential systems that govern the functioning of the organism, as well as to cover energy costs.

The concept of quality of meat and meat products is provided by a set of indicators. the main indicators are the biological value of the product, organoleptic indicators, hygienic and toxicological characteristics, as well as the stability properties.

The use of sodium nitrite (E250) in meat technology is provided by its complex effects on the quality of final products. Sodium nitrite contributes to the formation of color, is involved in the formation of taste and flavor of meat, it also inhibits the activity of microorganisms, the development of oxidative processes. the acceptable daily intake (mg/day) must not exceed 0.2 mg per day. in raw smoked sausages the sodium nitrite content must be not more than 0.003 % in the boiled, smoked and boiled smoked sausages — not more than 0.005 %; in sausage products meant for baby and dietary foods — 0.0015 %. Sodium nitrite is used as an additive in salting meat and meat products to save red colour. When salting meat red dye myoglobin, turning into boiling gray-brown metmyoglobin reacts with nitrite to form a red

nitrosomyoglobin. This compound, which gives meat products typical red salted meat is not changed on boiling and more stable than myoglobin, to the impact of atmospheric oxygen. the most optimal pH for the formation of nitrozomioglobin are from 5,2 up to 6,6[8].

The intensity and stability of the pink color of sausages are one of the main indicators of sausages' quality. Along with the stabilization of color, nitrites together with cooking salt have a preservative effect. They are used as a curing mixtures consisting of sodium chloride and sodium nitrite in an amount of 7,5 g per 100 kg of raw material. Sodium nitrite is recommended as a means of preventing the progress *Cl. Botulinum.*

The low levels at which nitrite occur in foods and the heterogeneous nature of foods necessitate the use of sensitive methods of analysis. These methods require several steps, e.g. isolation from the food, clean-up and concentration, separation, detection, and quantification.

Several methods are now available for the determination of nitrite in food stuffs, these have been reviewed. Among the methods more frequently used are spectrophotometry, chemiluminescence, gas chromatography (GC) and gas liquid chromatography (GLC) with fluorescence detection[9].

Spectrophotometric method is widely applied for the estimation of nitrites[10].

*The purpose of this work* is to determine the content of sodium nitrite in sausages and smoked swine products using the Griess method. Griess reagent (sulphanilamide and N-(1-naphltyl) ethylenediamine hydrochloride (NED)) in the presence of nitrite gives a red-pink

[8] *Rebwar O. H., Diyar S. A.* Determination of content levels of nitrogen species (Nitrite, Nitrate and N-Nitrosamine) in processed meat consumed in Erbil City // Der Pharma Chemica. 2010. Vol. 2, № 6. P. 31–37.

[9] *Encarnacion L. P., Angel R., Miguel V.* Fresenius Automated flow-injection spectrophotometric determination of nitrosamines in solid food samples // J. Anal. Chem. 2001. Vol. 371, № 6. P. 891–895.

[10] *Hitoshi K., Shigeo Y., Keiitsu S., Abena A.* Highly sensitive method for determination of N-nitrosamines using high-performance liquid chromatography with online UV irradiation and luminol chemiluminescence detection // J. of Chromatography A. 2009. Vol. 1216, № 18 2009. P. 92–98.

color of the solution whose intensity (absorbance) are determined by spectrophotometry.

Colouration of the solution is caused by the formation of azodye. the reaction proceeds in two stages.

The first reaction is the reaction of nitrite diazotizing sulfanylic acid in presence of acetic acid, and the second one is the reaction of the resulting product with α-naphthylamine. the latter reaction is slow, and the color will appear with time. These two reactions can be presented in Fig. 13.

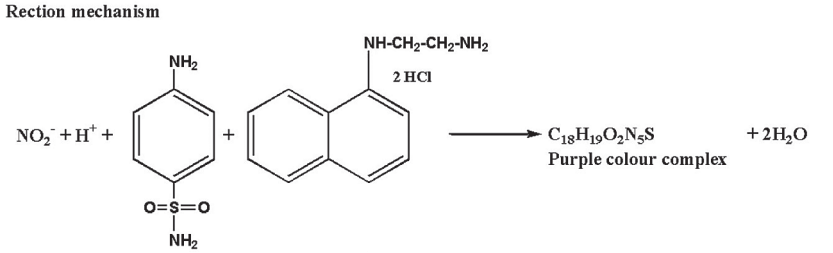



Fig. 13. The reaction mechanism of nitrite determination based on coloured azodye formation

## Experimental[11]

*Materials and equipment*

- Boiled sausage.
- Spectrophotometer Shimadzu UV-1800.
- Balances.
- Water bath.
- Volumetric flasks of 100 and 200 ml.
- Conical flasks at 100 and 250 ml.
- Beakers of 50 and 100 ml.
- Cylinders of 50 ml.
- Pipettes of 2; 5; and 10 ml.

[11] *Hitoshi K., Shigeo Y., Keiitsu S., Abena A.* J. of chromatography A, 1216. 2009. № 1. P. 92.

- Funnels.
- Glass rods.
- Paper filters.
- Cotton wool.
- Knives.

*Reagents*

- Griess reagent solution (10 g of Griess reagent was dissolved in 12 ml of concentrated acetic acid ($CH_3COOH$), and then 88 ml of distilled water were added).
- Sodium hydroxide, 0,1N solution.
- 0,45 % of $ZnSO_4$ was prepared by mixing 0,225 g of $ZnSO_4$ and 50 ml of distilled water. 10 ml of the solution were taken and heated in water bath for 10 minutes, after the content was added into a volumetric flask of 100 ml and then water was added up to the mark of 100 ml. Afterwards the solution was filtrated and stored in a bottle.
- Ammonia $NH_3$; 5 % solution.
- Hydrochloric acid HC1, 0.1 N solution.
- Sodium nitrite $NaNO_2$, standard solution (10 μg/ml).
- Distilled water.

*Preparation of Standard nitrite solution*

To prepare an initial 10 μg/ml $NaNO_2$ solution, it is required to weight 3.0 g of sodium nitrite and dissolve it in 50 ml of distilled water, then the mixture should be transferred quantitatively to 1000 ml volumetric flask, be shaken well and made up to the mark. Then 5 ml of this solution should be pipetted and added into 1000 ml volumetric flask and made up to the mark. 1 ml of this standard solution contains 10 μg of nitrite ion ($NO_2^-$). Then the standard solution prepared should be diluted to achieve the mass concentrations of $NaNO_2$ solution equal to 0.02; 0.015; 0.01 и 0.005 μg/ml.

*Extraction Procedure*

A piece of sausage is taken and ground into small particles using a grinder.

20 g of ground sausage were measured using balance, and then transferred to a beaker and 40 ml of distilled water heated at 55 °C are added, then the mixture is stirred using a mixer. Then the resulting mixture should be stood for 10 minutes at room temperature.

After 10 minutes the mixture should be filtrated using a Buchner filter covered with filter paper. 20 ml of clear extraction solution were taken and added into volumetric flask of 100 ml; afterwards 10 ml of 0.1 % NaOH solution and 40 ml of 0.45 % $ZnSO_4$ solution for protein deposition are added. the solution should be diluted to mark with distilled water and mixed.

*Determination of Nitrites*

5 ml of extracted solution (filtrate) or water solution and also blank or reference solution are transferred into a conical flask of 100 ml and 1ml of 5 % ammonium solution, 2 ml of 0.1N HCl solution and 5 ml of an initial 10 μg/ml $NaNO_2$ standard solution are added. the latter solution is added with the purpose of strengthening colouring. Finally, 15 ml of Griess reagent solution are added and the resulting mixture should be left for 15 minutes in the dark. Afterwards, the measurements of absorbance of the solution in a 1 cm cell using a spectrophotometer Shimadzu UV-1800 at wavelength of 520 nm should be performed.

*Calculations*

The absorbance readings obtained are directly related to the intensity of the color, which in turn is directly related to the concentration of nitrite present. the absorbance of the standard solutions provide a direct measure of the amount of nitrite present in the known solutions. By comparing the absorbance of the sausage extraction solution to the absorbances of the known concentrations, the amount of nitrite in the sausage solution may be estimated.

**Assignment**

1. Using any program of graphs visualization (i.e., Origin, Sigma Plot, Excel et al.), plot a *calibration curve of absorbance versus concentration*. the data obtained from the known solutions is used for this plot. Average the absorbance readings obtained for each of the known

concentrations. After plotting the points, draw a trend line ("line of best fit") through the points on the graph. If done correctly, your plot should produce a straight line that passes through the origin.

2. Use this graph and the absorbance of the sausage extraction solution to estimate the amount of nitrite present in it.

The mass concentration of sodium nitrite in a product is calculated by the formula[12]:

$$X = \frac{M_1 \times 200 \times 100 \times 28}{g \times 20 \times 5 \times 10^6}, \quad (5)$$

where $X$ is the the mass concentration of sodium nitrite in a product, %; $M_1$ is the mass concentration of sodium nitrite determined from the calibration plot, μ/ml; $g$ is the weight of a product, g; 28 — the volume of prepared colored solution, ml; 200 — the volume of extract, ml; 100 — dilution of extract, ml; 20 — the volume of extract taken for protein precipitation, ml; 5 — volume of filtrate to prepare the colored solution, ml; $10^6$ — Conversion factor (μg to g); 100 — Conversion to per cent.

Make a conclusion about quality of your sausage sample based on nitrites content.

**Questions**

1. What kind of effects on the quality of final products can be caused by using sodium nitrite (E250) in meat technology?
2. What are the main sources of nitrites, nitrates and nitrosamines intake in food stuff and edible raw materials?
3. What is the maximum allowable concentration for the raw and boiled smoked sausages?
4. Briefly describe the reactions which underlie the spectrophotometric method of nitrites determination in meat samples.

[12] *Hotchkiss J. H., Havery D. C., Fazio T.* Rapid method for estimation of N-nitrosodimethylamine in malt beverages // J. Assoc Off Anal. Chem. 1981. Vol. 64. P. 929–932.

# Laboratory Experiment 5. Refractometric determination of sucrose in sugar-containing drinks

## Backgrounds

Sugars are major sources of energy for all living entities. Plants produce sugars by photosynthesis and convert them into various disaccharides such as sucrose, or convert them into starch for easy storage. Herbivores make use of this energy source and are also attracted to the sweet taste and smell of sugars. Just because of the taste of sugar, humans have gone a step further, adding sugar to food that normally and naturally does not contain it, or has it only in small quantities. People now consume a great deal more refined sugar than their body weight allows. the human body cannot tolerate a large amount of refined carbohydrates, thereby damaging the vital organs. the average healthy digestive system can digest and eliminate two to four teaspoons of sugar daily, usually without noticeable side-effects. An excess of sugar in the diet results in weight gain, thereby increasing the risk of heart disease, diabetes and high blood pressure, apart from dental caries. It is therefore important to know what amount of sugar is present in food and beverages that are commonly consumed. Refractometry is one of the method which is used to determine the sugar (sucrose) content of syrups, fruit juices, ferments, vegetable juices, sugar-containing drinks and milk-based drinks, and to estimate the total concentration of monosaccharides and disaccharides in any solution[13].

## Experimental

*Equipment, chemicals and materials:*

- abbe refractometer IRF-454 (Kazan, Russia) is fitted with a scale giving either percentage by mass of sucrose to 0.1 % or refractive indices to four decimal places;
- the refractometer is equipped with a thermometer having a scale extending at least from +15 °C to +25 °C;

[13] Method OIV-MA-AS2-02: Evaluation by refractometry of the sugar concentration in grape musts, concentrated grape musts and rectified concentrated grape musts / Compendium of international methods of analysis-oiv, 2004.

- balances;
- volumetric flasks of 100 ml;
- cylinders and beakers of 50–100 ml;
- distilled water;
- samples for analysis;
- glass rods;
- filter paper.

*Refractometer zero point verification with distilled water*

It is necessary to make sure that the prisms and measuring cell are totally clean before the measurement.

1–2 drops of distilled water are placed on the lower prism of the refractometer, then the upper prism is drawn down and the measurement is conducted after 2–3 minutes. the border of light and shade should be clear and pass through the point of intersection of filaments (crosshairs) or a dotted line.

The refractive index of water ($H$) at 20 °C is equal to 1.3330. the temperature variations cause deviations in the refractive index of water indicated in Table 13.

*Table 13*

**Refractive index of water vs. Temperature**

| Temperature, °C | Refractive index of distilled water | Temperature °C | Refractive index of distilled water |
|---|---|---|---|
| 15 | 1.3335 | 23 | 1.3327 |
| 16 | 1.3334 | 24 | 1.3326 |
| 17 | 1.3333 | 25 | 1.3325 |
| 18 | 1.3332 | 26 | 1.3324 |
| 19 | 1.3331 | 27 | 1.3323 |
| 20 | 1.3330 | 28 | 1.3322 |
| 21 | 1.3329 | 29 | 1.3321 |
| 22 | 1.3328 | 30 | 1.3320 |

*Sample Preparation*

10 g of product is weighted with an accuracy 0.01 g, then it is transferred into a volumetric flask of 100 ml via a dry funnel as well as distilled water is added up to the level and the mixture should be agitated for 15–20 minutes.

*Measurement of sample refractive index*

Bring the sample to a temperature close to 20 °C. Place a small test sample (2–3 drops) on the lower prism of the refractometer, taking care (because the prisms are pressed firmly against each other) that this test sample covers the glass surface uniformly. Read the refractive index ($H_1$) to four decimal places. Carry out at least two determinations on the same prepared sample. Note the temperature t °C.

*Calculation of sucrose mass content in the sample*

The mass fraction of sucrose, $X_2$ %, is calculated by the formula (6):

$$X_2 = (H_1 - H) \times 10\,000 \times K, \quad (6)$$

where $H$ is the refractive index of distilled water at a temperature determination; $H_1$ is *the* refractive index of the test sample at a temperature determination; $K$ — conversion factor of the refractive index to the percentage of sucrose in the studied types of soft sugar containing drinks ($K$ = 0.2770). the final result should be the arithmetic mean of the results of two parallel determinations with the allowed difference between them not exceeding 0.3 %. Calculations are carried out with an error of no more than 0.01 %.

**Assignment**

Make a conclusion about sugar content using method of refractometry.

**Questions**

1. What the principle of refractometry underlies in sugar content determination?
2. Review the method of sugar content determination based on measuring specific gravity

# Laboratory Experiment 6. Determination of quinine in commercial soft drink by molecular fluorescence spectroscopy

## Backgrounds

Quinine is an alkaloid occurring naturally in the bark of trees or shrubs of the various species of two *Rubiaceous genera*, *Cinchona* and *Remijia*, indigenous to the higher eastern slopes of the tropical Andes in South America[14]. the medicinal properties of Cinchona bark were first recognized in the seventeenth century and until 1942, the sole source of quinine, a drug which was for years the only existing specific antimalarial remedy. in recent years however, another alkaloid quinidine, the stereoisomer of quinidine has been used in the treatment of heart conditions.

The use of quinine in carbonated mineral water began in the mid 1800's for combatting malaria. This practice is still continued today, though not for its antimalarial action, but as a beverage for the slightly bitter taste it imparts to the palate.

In this experiment, the technique of fluorescence spectroscopy will be utilized to determine the percentage quinine content in commercial samples of tonic water/bitter lemon[15]. Molecular fluorescence spectroscopy is based on the emission of light by molecules, which are excited to emit their characteristic spectra by exposure to UV light of specific wavelengths. the wavelength at which a molecule may be excited to emit is referred to as the excitation wavelength, and the spectrum emitted by the sample the emission spectrum[16]. Unlike UV-Visible spectroscopy, in molecular fluorescence spectroscopy, the emitted

[14] *Pelletier S. W., Reinhold N.* Chemistry of the Alkaloids. Cleveland. OH, USA: Zubal-Books, 1970. 800 p.

[15] Determination of quinine in commercial soft drink by molecular fluorescence spectroscopy : Analytical Chemistry Fluorescence spectroscopy of Quinine in Tonic Water. URL: http://delloyd.50megs.com/labscripts/fluores.html (Accessed: 15 January 2015).

[16] *Schulman S. G.* Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice. New York : Pergamon Press, 1979. 288 p.

spectrum is monitored at right angles to the exciting radiation, which renders the latter technique much more sensitive than the former. the intensity of emission at a specific emission wavelength is proportional to the concentration of the fluorescent molecule. This relationship forms the basis of quantitative analysis with this technique.

## Experimental

*Sample preparation*

- Use a 5 or 10 ml microburette and a 50 ml burette to prepare your standard and sample solutions. a standard quinine sulphate solution containing 10 mg of quinine sulphate in 1liter of distilled water will be provided.
- Use the microburette to transfer 0.5; 1.0; 1.5; 2.0 and 2.5 ml respectively of the standard quinine sulphate solution to 25 ml volumetric flasks, labeled 0.2; 0.4, 0.6; 0.8; and 1.0 ug/ml respectively.
- Using the 50 ml burette, add sufficient distilled water to each flask, so that the total volume in each is 12.5 ml, then fill each flask to the mark with 0,2N sulphuric acid, to give a final concentration of 0.1N acid for optimal fluorescence. Mix the solutions thoroughly.
- Obtain a sample of commercial tonic water and prepare a diluted sample as above. (See Demonstrator for dilution factor.)

*Molecular fluorescence spectroscopy*

The Perkin-Elmer LS5OB Luminescence spectrometer is an expensive, highly sensitive, research instrument. Under no circumstances should it be operated without proper supervision!!! the remainder of the procedure must be carried out under guidance from a demonstrator or technician:

- Rinse two matched cuvettes and fill one with the 0.5 ug/mL standard solution and the other with 0.1N sulphuric acid reference. *Avoid fingerprints on the cell faces!*
- Scan the emission range of the quinine solution, with excitation wavelength set at 350 nm. Determine and note the emission intensity value at the wavelength of maximal emission.

Similarly scan the blank solution, to obtain the blank emission value.

– Repeat measurements for all standard and sample solutions.

**Assignment**

- Plot the fluorescent intensity of each standard quinine solution, corrected for blanks, vs concentration.
- Determine the mean quinine (sulfate) concentration and Std. Deviation of the sample.

**Question and Exercise**

1. Why is fluorescence spectroscopy far more sensitive than UV-Vis spectroscopy?

2. Two fluorescent compounds A and B are present in a mixture. Using a spectrofluorimeter similar to the one used above, how would you determine the quantity of a in the mixture, if a absorbs radiation in a region where B does not. Both A and B absorb in the same spectral region, but a emits fluorescence at different wavelengths to B.

## Laboratory experiment 7. Microbial analysis of food stuffs

**Backgrounds**

Microorganisms (microbes) are a large group of mostly single-celled organisms, which can be distinguished only under the microscope, with the characteristic size of less than 0.1 mm and are organized easier than plants and animals.

Microorganisms which capable to cause infections in humans and animals are called as pathogens (hazardous under any conditions) or opportunistic (dangerous under certain conditions). Opportunistic pathogens such as *Escherichia*, *Proteus, Klebsiella, Enterobacter, Serratia et al*. become pathogenic only when ingested in large amounts or penetrate into a weakened body. Pathogenic organisms cause disease in humans and animals and plants. Other microorganisms cause the loss of agricultural production, leading to depletion of soil nitrogen, result

in water pollution, the accumulation of toxic substances (e.g. microbial toxins)[17].

Microorganisms that are permanent inhabitants of the surfaces and cavities of human or animal body, are called sanitary-indicative microorganisms. They can be found in the environmental objects, contaminated secretions, as well as in human or animal body. the pollution is more abundant, the greater the possibility of entering opportunistic and pathogenic microbes an object.

The favorable range of temperatures for the active microorganisms propagation is 20–37 °C, therefore meat, fish, milk and other products in warm room conditions becomes perishable very soon. Various products contain different amounts of pathogenic and opportunistic microorganisms. in order to bring nutrition into a healthy state it is necessary to choose the right products and to know the conditions of their storage and preparation. Therefore, it is very important to know which food stuff has a high content of microorganisms both pathogenic and opportunistic[18].

*The purpose of the laboratory work* is to determine the number of colonies of various types of microorganisms in the test food stuffs and to identify which food stuffs are the most and least contaminated by microorganisms.

**Experimental**

*Objects:* boiled eggs, raw chicken, raw beef, bread.

*Equipment, reagents and materials:*

– peptone water;
– agar nutrient media;
– sterile container;
– tubes.

*Procedure*

- Fill agar culture medium into 4 Petri dishes (number of test samples), give some time to cool down.

[17] *Taylor J.* Microorganisms and Biotechnology. Nelson Thornes, 2001. 184 p.

[18] *Surajit Das, Hirak Ranjan Dash*. Microbial Biotechnology. A Laboratory Manual for Bacterial Systems. Springer, 2015. 239 p.

- Prepare peptone water (1 g of peptone, 0.5 g salt per 100 ml water). Sterilize 15 minutes under a boiling water bath.
- Prepare the solution of the food product: 0.01 g of product is dissolved in 50 ml of peptone water.
- Make bacterial inoculation in Petri dishes with microbiological loops flambéed in flame.
- Cover plates and flip them. Microorganisms colonies are grown in an incubator at 37 °C for 6 days.
- Count colonies of microorganisms using a counter, determine their types and characteristics.

**Assignment**

Make a conclusion about food stuffs which the most and least contaminated by microorganisms.

**Questions**

1. What kind of the microorganisms are included in hygienic standards on the base of microbiological indicators?
2. What is the difference between pathogenic and opportunistic microorganisms?
3. Why peptone water is used for food sample solution preparation?

## Laboratory Experiment 8. Spectrophotometric Determination of Iron in Different Types of Flours

**Backgrounds**

Selectivity of the methods to determine iron will depend whether total iron or iron from a specific source of iron is measured.

Quantitative methods to determine iron are highly selective and visible spectrophotometry or atomic absorption spectrophotometry (AAS) are used.

This procedure contains visible spectrophotometric method, but once the sample solution is prepared after ashing, the solution may be used either be treated with the chromogenic agents or used for analysis by AAS. the choice of method will depend on the availability

of equipment in the laboratory. the AAS method may be faster than the spectrophotometric alternative, but the general disadvantage of AAS is that it will determine total iron, regardless of its salt of origin if the sample is ashed.

Special extraction procedures allow extracting ferrous iron from the sample and determining it by visible spectrophotometry.

The determination of total iron in food usually includes the total combustion of organic materials leaving only the ash, which contains the mineral part of foods. This process transforms all iron present to the oxidized ferric form ($Fe^{3+}$). a solution of the ash is prepared using hydrochloric acid and the iron(III) iron is reduced to iron(II) using hydroxylamine hydrochloride.

The ferrous ion ($Fe^{2+}$) can be determined spectrophotometrically by forming colored complexes using several chromogens that interact with iron ($Fe^{2+}$) such as 1.10-phenanthroline $\cdot H_2O$; bathophenanthroline, (a disulphonic salt of 4,7-diphenyl-1,10 phenanthrolyne); $\alpha,\alpha$-dipyridile (2,2' bipyridine); or ferrozyne (acid[3-(2-pyridyle)-5,6-bis-(4-phenylsulphonic)-1,2,4-triazine). The color reaction has to be performed under pH-controlled conditions suitable for the chromogen. in order to reduce the competition by hydronium ions ($H_3O^+$) for the ligand, a solution of 2 M sodium acetate is added[19].

## Experimental

*Equipment and Materials*

- Scanning Spectrophotometer UV/VIS Camspec M501;
- Secondary Standard Solution of Iron-10 mg/l;
- Standard Solutions for the Calibration Curve with concentrations of iron from 0 to 5 mg/l (ppm);
- Glass cuvettes for spectrophotometric measurements;
- Solutions of wheat, corn and soya flour ashes.

[19] Manual of laboratory methods for fortified foods (Vitamin A, Riboflavin, Iron and Iodine), 1st Edition / ed. by M. Guamuch, Ph. Makhumula, O. Dary. Kampala, 2007. 64 p.

*Preparation of Flour Ash Solutions*

The ash is evaporated by heating the crucibles on is dissolved by adding 2 ml of concentration HC1, and heated for few minutes. the crucibles with samples solutions were cooled down and the solutions were quantitatively transferred into a 25 ml volumetric flask. the flasks were made up to volume with deionized water.

*Preparation of Standard Solutions*

**Primary Standard Solution of Iron — 1000 mg/l**

Dissolve 3.512 g of $Fe(NH_4)_2(SO_4)_2 \times 6H_2O$ in distilled water, and add a few drops of concentrated HCI. Dilute to 500 ml in a volumetric flask. Transfer the solution to a plastic bottle. This solution is stable for indefinite time, unless a light pink color is observed indicating contamination.

**Secondary Standard Solution of Iron-10 mg/l**

Into a 500 ml volumetric flask pipette 5 ml of the Primary Standard Solution (1000 mg/l). Add 2 ml concentrated HCl. Fill with distilled water up to the 500 mL mark. Transfer the solution to a plastic bottle and store it in a cool dry place. This solution is stable for about 6 months.

*Preparation of Calibration Curve*

9 standard solutions for the calibration curve with concentrations of iron 0 (1); 0.2 (2); 0.5 (3); 1 (4); 1.5 (5); 2 (6); 3 (7); 4 (8) and 5 mg/l (ppm) (9) are made using the following procedure.

Into 100 ml volumetric flasks, the amounts of the Secondary Standards Solution (10 mg/l) are pipetted (They are specified in the Table 14 below). Then flasks are made up to volume with deionized water. Then the solutions are mixed thoroughly by inverting the flask several times and transferred the into properly labeled plastic bottles. These standard solutions are stable for approximately six months.

*Determination of Iron*

**Pre-preparation**

10.0 ml of the sample solution are pipetted into 25.0 ml volumetric flask, then 1.0 ml of hydroxylamine hydrochloride solution is added. the solutions are mixed well and stood for 5 minutes. With

the standards, 10.0 ml of the standard solutions prepared in above are pipetted into 25.0 ml volumetric flasks, but do not add hydroxylamine hydrochloride solution. Then 5.0 ml acetate buffer and 4.0 ml of 1.10-phenanthroline were added to each flask. All solutions were mixed well and color is started developing. They stood for 30 min and then the flask are made up to volume (25 ml) using deionized water.

*Table 14*

**The amounts of the Secondary Standards Solutions (10 mg/l) to be added to reach the specific iron concentrations**

| Iron, mg/l (ppm) | Volume of the secondary solution (10 mg/l) to be added (ml) |
|---|---|
| 0.0 | 0.0 |
| 0.2 | 2.0 |
| 0.5 | 5.0 |
| 1.0 | 10.0 |
| 1.5 | 15.0 |
| 2.0 | 20.0 |
| 3.0 | 30.0 |
| 4.0 | 40.0 |
| 5.0 | 50.0 |

*Spectrophotometric measurements*

- Turn on the spectrophotometer 15–20 minutes before using it to warm up;
- Adjust the wavelength to 510 nm;
- Set the mode to Absorbance;
- Set the instrument to zero Absorbance using deionized water;
- Read the absorbance of the 0.0 mg/l standard solution (1) (blank) and record the absorbance;
- Read the absorbance for the standard solutions and flour sample solutions.
- If color intensity of the samples is too high, make appropriate dilution of the sample solutions and record the absorbance again;

- Plot a graph of the absorbance values of the standard solutions ($y$-axis) against concentration ($x$-axis) and determine the concentration of iron in the flour sample as described in the section on calculations.

## Assignment (Calculations)

1. Calculate the regression line of absorbance ($y$) versus iron concentration (Fe) in mg/l ($x$), as presented in the following equation:

$$y = m \times x + c, \tag{7}$$

should be 0.99 or bette

(for plotting a graph it is necessary to use Excel, Origin, Sigma Plot or any other software available).

2. Calculate the iron concentration of the sample (mg/l) ($x$) directly from the regression equation, substituting the value of ($y$) with the absorbance of the sample.

$$x = (y - c) / m. \tag{8}$$

3. In order to report the content of iron in mg of iron per kilogram of food, multiply the previous result as follows:

$$C_{Fe^{2+}} = \frac{[Fe] \times V_i}{w}, \tag{9}$$

where $C_{Fe^{2+}}$ and [Fe] are the iron concentrations in mg/kg and in mg/l based on the calibration curve; $V_i$ is the volume of the initial solution, ml ($V_i$ = 25 ml); $w$ is the weight of the sample, g ($w$ = 1 g).

The equation can be simplified as:

$$C_{Fe^{2+}} = \frac{[\mathrm{Fe}] \times 25}{w}.$$

Make a conclusion about the iron concentration of different types of flour. Which type of flour has the highest iron concentration?

# RECOMMENDED LITERATURE AND RESOURSES

American Society of Testing Materials/ astm.org. URL: http://www.astm.org/cis/en/index.html/ (Accessed: 19 December 2015).

Codex Alimentarius Commission / codexalimentarius.org. URL: http://www.fao.org/fao-who-codexalimentarius/en/ (Accessed: 19 December 2015).

European Committee for Standartization/ cen.eu. URL: https://www.cen.eu/Pages/default.aspx/ (Accessed: 19 December 2015).

Food and Agriculture Organization of the United Nation/fao.org. URL: http://www.fao.org/home/en/ (Accessed: 19 December 2015).

International Organization for Standardization/ iso.org. URL: http://www.iso.org/iso/home.html (Accessed: 19 December 2015).

Food safety from farm to table. a national food-safety initiative // a report to the president. Environmental Protection Agency, Department of Health and Human Services, US Department of Agriculture, Washington, DC, 1997. 58 p.

USDA (2002). General specifications for dairy plants approved for USDA inspection and grading service // Dairy Program, Agricultural Marketing Service, US Department of Agriculture, Washington, DC/ usda.gov. URL: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004788 (Accessed: 19 December 2015).

USDA (2005). Milk for manufacturing purposes and its production and processing, recommended requirements // Dairy Program, Agricultural Marketing Service, US Department of Agriculture, Washington, DC/ usda.gov. URL: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3004791.

USDA (2006). Commodity specification of all-purpose egg mix. September 2006. Poultry Division, Agricultural Marketing Service, US Department of Agriculture, Washington, DC/ usda.gov. URL: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5048747.

USDA (2008) Commercial item description. Tea, instant. A-A-220183C. 21 Oct 2008. General Services Administration, Specifications Unit, Washington, DC/ usda.gov. URL: http://www.dscp.dla.mil/subs/support/specs/cids/20183.pdf.

Food Science Experiment and Activity Guide (electronic text book) / Home Economics Education, North Carolina Department of Public Instruction, Raleigh, North Carolina, 1994. 160 p.

*Roday S.* Food Science and Nutrition / S. Roday. Oxford University Press, London, 2012. 428 p.

# ANNEXES

## Annex 1

## REPORT

I have studied the following standard (wright down a standard which is given to you by an instructor) _____

_____

*Table A1*

**Clauses of standard vs. Sub-Clauses**

| Clauses of the standard | Sub-clauses (if any) of the standard and the main content thereof |
|---|---|
| Scope | |
| Definition of Produce | |
| Essential Composition of Quality Factors | |
| Quality | |
| Sizing | |
| Tolerances | |
| Presentation | |
| Marking or Labeling | |
| Contaminants | |
| Hygiene | |

## Annex 2

# SAMPLING PROCEDURE

**Sampling**

Relevant conditions/circumstances (temperature/humidity)
Preservative substance
Sampling equipment sterilized by
□ Sampling person □ Laboratory sampling □ Others
method (tick all that apply, note differences) (см. Fig. A1).

**Sampling details**

Did the samples include the smear? _____□ yes □ no
Did the samples include the rind? _____□ yes □ no
If no, how many millimetres of the rind were cut off?
Are the samples grated? _____□ yes □ no

**Sample preparation (preparation of test portion)**

How were the above-characterized samples prepared for analyses?
□ Including the surface
□ Excluding _____mm of the surface, this equals % mass removal of the original sample
□ Grating the samples, using the following equipment:
□ Other treatments, namely _____

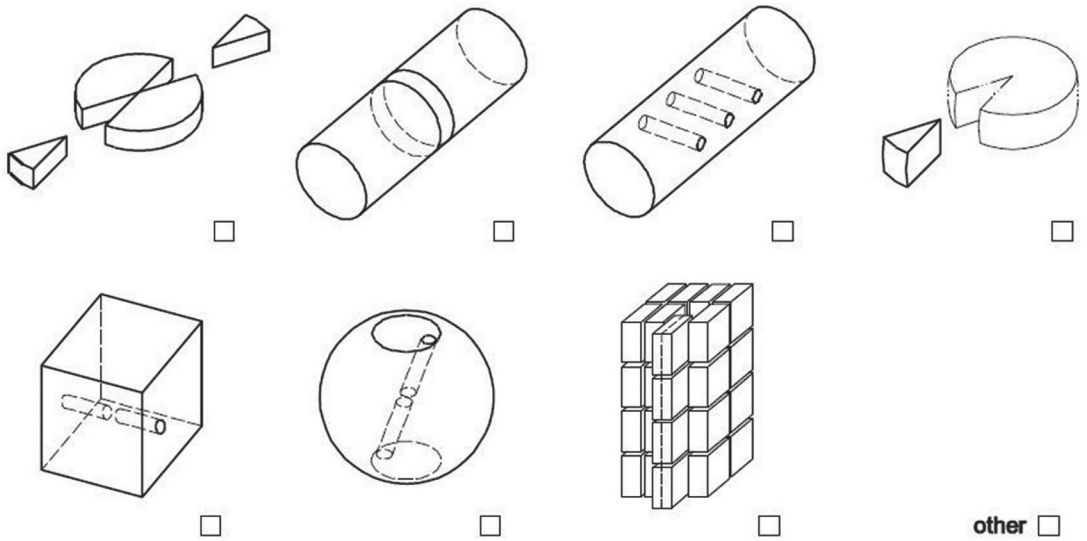



Fig. A1. Schematic procedure of Sampling

**Annex 3**

## SAMPLING REPORT FOR CHEESE

This sampling report is for cheese only but can be used as a model for other dairy products

**Sample**

Identification no _____
Sample description _____
Batch/code/marking _____
Date of expiry _____
Cheese variety/age _____
Cheese surface (tick all that apply)
□ rindless □ rindn □ rind with smear □ coating □ type of coating
No. samples_____
Sample mass (approximate) _____
Packaging □ prepacked □ aluminium □ plastic
(tick all that apply) □ vacuum □ modified atmosphere
Origin
Place _____
Date_____
Location _____
(name/address of producer/ trader/packaging)
Time of sampling (facultative) _____
Laboratory/place to be sent_____
Name of sampling person _____
Designation of sampling person _____
Signature_____
Name of witness _____
Designation of witness _____
Counter signature _____

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# БЕЗОПАСНОСТЬ И КАЧЕСТВО ПИЩЕВЫХ ПРОДУКТОВ

# PRACTICAL FOOD SAFETY AND FOOD QUALITY

Практикум

На английском языке

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Факс: +7 (343) 358-93-06
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